

DDT may be Good for People

DR N. E. BORLAUG is entirely right to protest, as he did in Washington last week, that too zealous a campaign to regulate the use of DDT, and in particular a ban on it, will do much more harm than good. He might have added that it is an immense irony that a comparatively simple chemical widely regarded twenty years ago as a great benefaction has almost become a symbol of all things fearsome. Part of the trouble is that DDT and the other organochlorine pesticides such as dieldrin and aldrin were the centrepiece of Miss Rachel Carson's book *Silent Spring*. In some atavistic way, however, pillorying this chemical serves to satisfy a great many vague but common fears—that governments are incompetent in regulating the uses of innovation, that greedy manufacturers are urging farmers to use more of these materials than is strictly necessary, thus demonstrating yet another way in which the search for private profit will undermine society, and also, given the apparent ubiquity of DDT in the environment, that it is never possible in the modern world to escape from the secret threat of technology, the centre of fashionable paranoia. It is regrettable that in the past few years, professional scientists have done as much as anybody to lend support to what has become a thoroughly irrational protest against the persistent pesticides. It would be a great public service if some members of the profession were to respond to Dr Borlaug's plea by helping to put the dangers of DDT in some kind of a perspective.

If many facts are still uncertain, it is at least possible to say what the uncertainties consist of. Since DDT was first introduced to agriculture and public health immediately after the Second World War, there have been many occasions on which this and other persistent pesticides have been misused. Sometimes excessive doses have been used, so that animals have been killed off as well as insects. Sometimes, so little has been understood of the natural pressures to which a pest is subject that DDT and its relations have killed not the pests at which they were directed but their natural predators, with entirely unpleasant results. And there are always circumstances in which the organochlorine pesticides can be concentrated at the top of a food chain, thus imposing a strain on species quite unrelated to pests.

For reasons like this, there is a strong case for better methods of regulating the application of pesticides so as to ensure that their use in agriculture or public health does not bring about unwanted side effects. Even if this should imply that the modern pesticides are too potent to be left in the hands of farmers and others without professional training, as the United States Administration's new proposal for legislation would suggest, that would not be an insuperable obstacle to the successful use of these materials. It is, however, quite another matter to suggest a more general restriction of the use of DDT and related

chemicals as pesticides. That might be throwing out the baby with the bath water.

In spite of a decade of research, there is at present only enough evidence to justify an agnostic position on DDT. Nobody should complain, for the problems have turned out to be unexpectedly difficult. It is, after all, worth remembering that the persistent pesticides are by their nature chemically inert, so that analysis must depend on physical methods such as chromatography, mass spectrometry and spectral absorption. The most headway has been made with gas-liquid chromatography, but only recently has it been appreciated that the polychlorinated biphenyls are easily confused with the pesticides proper. To be sure, discriminating practitioners know that a sequence of otherwise inexplicable peaks in a chromatography plot may be indicative of a mixture of biphenyls and pesticides, but less careful workers, or earlier workers, have often ignored such evidence. The result is that many of the measurements in the early sixties of concentrations of DDT in the environment may be misleading. Although there is no doubt that substantial quantities of the pesticides such as DDT are to be found in the flesh of fish and seabirds and in the fatty tissues of mammals, human beings included, there is at present no means of being sure whether the organochlorine pesticides are distributed on a truly world-wide basis or whether their spread is more a regional or even a local phenomenon, a measure of agricultural practice in the neighbourhood. In the past few years, it does appear that concentrations of DDT in human fatty tissue are more than twice as great (twelve parts per million) in the United States as they are in Britain. For what it is worth, the reports of DDT in the tissues of Antarctic penguins, portentous though they may be, refer to proportions which are smaller by a factor of ten or more. What this implies is that there may be a high degree of correlation between the use made of DDT in some agricultural region and the proportions which find their way into the fatty tissue of the people eating the food produced there. Before too many breasts are beaten on the altar of global pollution, it would be helpful if there were a better understanding of the distribution of these pesticides.

The great uncertainty which persists about the effects of DDT on human beings is another question that needs to be resolved. In these heady days of anxiety about the environment, it tends to be forgotten that only twenty-five years ago DDT was welcomed because it was effective against insects and much less harmful to people than other insecticides then in use. To be sure, DDT itself is poisonous in sufficiently large doses and its relatives are usually more toxic still, so that there is already a long and depressing record of death by accidental poisoning. On the other hand, no serious harm seems to have come to those who work in factories where pesticides are manu-



factured and who have avoided acute toxic doses. Careful studies have shown, with samples which are admittedly small, that working in pesticide factories brings neither an increased risk of liver disease nor an increased risk of cancer. So what of the hazards of the small quantities of these pesticides now stored in fatty tissues? The simplest statement of the position is still that of the Mrak Commission which said in December 1969 that "the only demonstrable effect of this exposure in man is the tissue residue". For practical purposes this view was confirmed last month by the United States Environmental Protection Agency's DDT Advisory Committee (see *Nature*, 233, 299; 1971) which said that "DDT has a very low acute toxicity to man and his domestic animals, and exposure to high doses for short periods of time does not appear to cause any irreversible damage". The same committee goes on to say that the risk of carcinogenesis is low—the only suggestions so far of such effects are a series of experiments with laboratory rodents. Clearly, the danger that DDT and its relatives may damage human beings is exceedingly small in spite of the profligacy of the use of DDT in the years immediately after the Second World War.

So what is the case for banning the use of DDT? Other species than human beings are the only causes that carry weight. Careful experiments with birds in captivity have shown that DDT can make eggshells thinner and thus impede the hatching of young birds. Such effects may well be responsible for the reduction of pelican populations in the Mississippi Delta and off the west coast of the United States in the past few years, but it remains a sobering truth that more eagles have been shot by farmers than can possibly have been killed by DDT. Outside the United States, it remains to be seen whether changes in the sizes of bird populations are to be explained by pesticides in the diet, by other causes such as the urbanization of the countryside or simply by changes in

the vigilance of bird watchers. On balance, however, it does appear that the damage so far done to wild life, while important and regrettable, is not disastrous and is easily attributable to extravagant use of DDT in the United States in the late fifties and early sixties. To be sure, there are more remote dangers to be considered—the possibility that pesticides might affect the metabolism of phytoplankton and thus exert an influence on the productivity of the oceans, but more needs to be known of the processes involved before anything but a hypothesis can be put forward.

In all the circumstances, there is a danger that speculation, sometimes quite proper speculation, about the effects that pesticides might have will be dignified by the status of received doctrine. The difficulty is that DDT and many of its works are economically important and, as Dr Borlaug points out, have a specially important part to play in helping to make sure that food production in the world will rise more quickly than the population. This is why it is important that the present wave of sentiment in favour of a ban on the use of DDT and such materials should be replaced by a proper and necessary case for regulation and supervision of the ways in which these pesticides are used. At the same time, of course, it is essential that there should be a better understanding both of the mechanics of the distribution of DDT, the mechanism by which it is accumulated in human tissue and the damage which it is likely to do to human beings. One absorbing question, for example, is why there should be such a large spread of DDT concentrations in human fat about the average value. University research could have an important part to play in furthering this understanding, for governments and industry have axes of their own to grind. But professional scientists everywhere could help by insisting that the present wave of discussion about DDT and other pesticides is conducted with a better sense of proportion than in the past.

Where are the Bounds of Decent Practice?

THERE have in the past few days been allegations that the experiments carried out in the past eleven years at the University of Cincinnati have offended against one of the essential principles of medical research—that human beings should not be used as experimental subjects without volunteering for the role and without being fully aware of all the circumstances. In all the discussions that there have been in the past few years of the problems of medical ethics in laboratory situations, this principle has been repeatedly affirmed. It is fair to say, of course, that the principle by itself is insufficient. What precisely is intended by the injunction that a potential experimental subject should "understand"? How can laymen hope to appreciate all the subtleties of a medical experiment, even if they grasp the essential objectives? And there are also difficulties in knowing what should be done in circumstances in which people may be tempted to volunteer in ignorance or under pressure (as when participation in an experiment carries with it the promise of early parole from prison).

It would be in the public interest that there should be an investigation into the circumstances in which the Department of Defense let a contract to the College of Medicine at the University of Cincinnati and the circumstances in which this has been administered in the past eleven years. Whether Senator Kennedy's congressional committee is the best forum is another matter. The hope now is that extraneous issues will not confuse the overriding question of how human experimental subjects should be dealt with and whether on this occasion they have been dealt with wrongly.

One obvious irrelevancy is the involvement of the Pentagon. No doubt there are many people who will say that it is in all circumstances reprehensible to use animals, human or otherwise, in experiments which are preparations for some nuclear battlefield. This, however, is entirely beside the point. So long as the military forces of the United States, the Soviet Union and Western Europe are organized so as to defend themselves against nuclear attack, it is

clearly unreasonable to expect that the services responsible will refrain from taking steps to know what doses of radiation soldiers can withstand. Those who say that the College of Medicine at the University of Cincinnati should not have accepted a contract from the Department of Defense on grounds like these have no logical alternative but to spend their energies on a refutation of the defence policy to which the governments of the principal powers are committed. But that is a wider issue, not relevant to what is said to have been happening at Cincinnati.

It is also beside the point to complain that therapy should also be used as an occasion for research. In medical research, there is also a long and heroic tradition of willingness on the part of one generation to endure discomfort voluntarily for the sake of a better understanding of some condition or disease. Patients with terminal disease have frequently been concerned with such experiments and, such is the inherent altruism of many people, have participated willingly. But it is clear that the dignity of everybody concerned, and that of the profession of medical science, demands that there should be a full disclosure of all the circumstances to those who are asked to participate. If one objective of a study is to make it easier to know what doses of radiation can be tolerated, by troops or by civilians, it is obviously incumbent on the people in charge to help their patients to understand by giving not merely a laconic description of the purpose of the experiment but also an account of the ways in which the information is likely to be valuable. If the Pentagon is a sponsor of the work, this should be spelled out.

From the reports so far available, it is not clear how far these conditions have been met, although some of those concerned have said that patients were told that "this research will be valuable if somebody is injured on the battlefield". What will need to be determined now is whether it is a sufficiently large step. An especially taxing feature of the Cincinnati case is the allegation by the *Washington Post*—it is no more than that—that many of the patients in the experimental series were in some way or another less able than they might have been to appreciate what was being asked of them. Circumstances like these are necessarily hard to reconcile with the essentials of what should be the code that governs the relationship between a patient-subject and his doctor-experimenter, but the only workable rule is that if a prospective subject is thought to be incapable of understanding fully what is expected of him, he should not be involved. As yet, there is nothing to suggest that the people at Cincinnati have not behaved with great propriety. It is, however, essential for everybody, and for medical research as such, that this should be demonstrated clearly and publicly.

One Way to Europe

WITH the British political parties preoccupied with their invariably introspective party conferences at British seaside towns (Brighton this year), and with the question of whether the British Government will eventually accept the terms of membership offered by the European Communities reduced to questions of which party leader will be able to carry how many of his supporters with him, the

initiative for taking a few practical steps towards Europe has fallen to others. And as luck will have it, there is an obvious and urgent task for universities in Britain and on the mainland. Will they dare?

In the past few months, there have been several declarations of how necessary it will be, once the European Communities are enlarged, for there to be a coordination of European policies on research and development. Because development is necessarily linked closely with industry and with commercial interests, the best place to start devising machinery for collaboration is in basic research, in the universities and government laboratories. This point was put forcefully by Sir Brian Flowers, chairman of the Science Research Council, at the British Association meeting in September. One difficulty is that the coordination of research between European countries entails the free movement of people between one laboratory and another. But there are at present practical difficulties which prevent this happening. And so is there not a case for a serious attempt by European universities to work out among themselves some method of doing this job? On this occasion, as luck will have it, they could act more quickly than their governments.

100 Years Ago



Extract from Helmholtz on the Axioms of Geometry.

With all due deference to so eminent a man as Helmholtz, I must hold that his article includes an *ignoratio elenchi*. He has pointed out the very interesting fact that we can conceive worlds where the Axioms of our Geometry would not apply, and he appears to confuse this conclusion with the falsity of the axioms. Wherever lines are parallel the axiom concerning parallel lines will be true, but if there be no parallel lines in existence, there is nothing of which the truth or falsity of the axiom can come in question. I will not attempt to say by what process of mind we reach the certain truths of geometry, but I am convinced that all attempts to attribute geometrical truth to experience and induction, in the ordinary sense of those words, are transparent fallacies. Mr. Mill is another philosopher whose views led him to make a bold attempt of the kind. But for real experience and induction he soon substituted an extraordinary process of *mental experimentation*, a handling of ideas instead of things, against which he had inveighed in other parts of his "System of Logic." And the careful reader of Mr. Mill's chapter on the subject (Book II. chapter 5) will find that it involves at the same time the assertion and the denial of the existence of perfectly straight lines. Whatever other doctrines may be true, this doctrine of the purely empirical origin of geometrical truth is certainly false.

W. STANLEY JEVONS

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OLD WORLD

TRADE UNIONS

Enter the APST

ONE of the first bodies to apply for registration under the Industrial Relations Act at the beginning of October was the Association of Professional Scientists and Technologists, which was formed recently by the Council of Science and Technology Institutes. When the council itself was set up in 1968 by five professional scientific institutes (including the Institute of Physics and the Royal Institute of Chemistry), its principal aim was to put forward the views of its member institutes on such subjects as the training of technicians and teachers; but when it became clear in the summer of 1970 that the government's legislation on industrial relations could pose problems for professional scientists, the council set up a working party to examine the desirability of forming an independent protective association.

Although the Industrial Relations Act does cater for professional bodies to a certain extent—they can be included on a special register and thus enjoy some of the rights of trade unions, for example—many of the professional organizations find their hands tied in other ways. Activities by the science institutes in the financial interests of their members are restricted to varying extents by their charters or articles of association, and separate salary negotiations for scientists in different disciplines would anyway be unacceptable both to employers and to employees.

The association is at present run by staff from the council's member institutes and the principal permanent staff will not be appointed until early 1972 when the association hopes to have a permanent headquarters and a healthy membership. The council says that between 5,000 and 10,000 members will have to be recruited during the next month or two so that the association can make itself felt before employers become too involved with the intricacies of the Industrial Relations Act. The annual subscription for a full member will be £5.

Dr Louis Cohen, secretary of the Institute of Physics, said this week that the association would probably attract principally those scientists who work in industry; he estimates, for example, that there are 4,000 to 5,000 potential members of the association in the Institute of Physics.

The Council of Science and Technology Institutes is conscious that, especially outside the industrial sector, some groups of scientists (for example, those in the civil service) already have effective negotiating bodies and that

"there is no question of trying to usurp their position in any way". Mr William McCall, general secretary of the Institution of Professional Civil Servants, says that competition for members between the association and the institution is unlikely and that it would anyway be self-defeating.

EUROPEAN LABORATORY

Vain Effort?

A CAMPAIGN to avert the threatened closure of the European Space Research Institute (ESRIN) is gathering momentum as a result of a conference of the Plasma Physics Division of the European Physical Society held at ESRIN in September. A united front was formed against the proposed closure under the leadership of Professor B. Lehnert of the Royal Institute of Technology, Stockholm, chairman of the Plasma Physics Division.

The decision to close the laboratory was made at a meeting of the council of the European Space Research Organization in July. The chairman, Professor G. Puppi of Bologna, proposed a plan for ESRO which was adopted by the council and which recommended that "circumstances enforce consideration of closing down ESRIN" but "the council would welcome any alternative viable solution". This decision is to be ratified at a further meeting of ESRO in November.

Professor Lehnert said this week that one particularly distressing feature of the affair was the lack of warning given to the scientific community of the closure. The presentation of the *fait accompli* has undermined the morale of the laboratory and, even if the laboratory can still be saved, the damage is probably irreparable. Lehnert, on behalf of the Plasma Physics Division, has this week sent a letter to ESRO protesting against the decision. He stresses that the laboratory has made several contributions to plasma physics and "during the existence of ESRIN, members of the [Plasma Physics] Division have established contacts with the staff and realize the high value of the scientific work performed at ESRIN". The letter continues, "The ESRIN laboratory must be considered an essential and important part of activities within ESRO and not as a luxury. We consider the proposal to relinquish ESRIN a retrograde step in European space research. Any apparent saving in the budget will be nullified by a decrease in the efficiency of ESRO and by increased pressure on national research budgets." It is very difficult to argue with this logic and an ESRO spokesman did not attempt to deny it when he said this week that ESRIN could not carry on in its present form because of financial

problems. He said, "ESRO is entering the applications field—air traffic control, meteorology and telecommunications—and some of the money now being spent on scientific programmes must go. The programmes least connected with essential ESRO activities—the ones at ESRIN and the rocket sounding programme—are being run down."

The reason for the precipitate decision to close the laboratory has aroused speculation in the European press in recent weeks. An article in *Die Welt* on September 30 says that the decision was forced upon ESRO by a French threat that they would withdraw from ESRO if the laboratory was not closed. Professor Lehnert said that he did not know why the decision had been taken but he was certain that pressure was being exerted on ESRO from a member country. It is known that the French feel that the science budget of ESRO should be limited.

The efforts to save the laboratory within ESRIN seem doomed but the scientists hope that the laboratory can be maintained in its present form under the auspices of some other European or Italian organization. An ESRO spokesman said on Monday that the director general, Dr A. Hocker, is in touch with Italian organizations to find a solution. He said, "The Italian authorities accept the idea that the present ESRIN activities cannot continue permanently within ESRO and the director general is actively negotiating to see what activity could be carried out at ESRIN."

A problem with transferring the laboratory to Italian hands is the current unrest among Italian scientists. At present there are unfilled positions in Italian national laboratories and another addition to their number will probably not be welcomed by the Italians. A more feasible possibility, if the laboratory is to be maintained in its present form, is for another European organization to take ESRIN under its wing. EURATOM seems the obvious candidate although CERN is a possibility. A difficulty that the scientists are facing in their campaign is that ESRIN is not unique in Europe. There are several national laboratories that rival ESRIN in quality of work and so the motivation for keeping the laboratory as a viable entity is not so strong as it might be.

It is still possible that ESRIN will remain within ESRO but with a different purpose. The ESRO office in Paris said on Monday that the director general is now negotiating with the Italian authorities to see what activity could be carried out at ESRIN instead of plasma physics. There is no information on what this new purpose might be but a spokesman said that it would be more closely related to the activities of ESRO than previously.

INSECTICIDES

Pyrethrin Prospects

ONE of the most promising developments in pesticides in these days of great public concern for the environment has been the development and marketing of pyrethrin analogues. Pyrethrum, a natural pesticide from the ground flowers of *Chrysanthemum cinerariaefolium* and *C. coccineum*, has been known since the early nineteenth century; six esters are found in the pyrethrum of which the most important and plentiful are known as pyrethrins I and II. They are used against houseflies, mosquitoes and cockroaches, mainly in the domestic field; their great advantage is that pyrethrin I has a high kill rate and pyrethrin II a high knock-down rate and that, once used, they degrade quickly in the light, leaving no appreciable toxic residues.

Pyrethrum has never been cheap; today a kilo of 100 per cent active pyrethrum costs about £38 compared with about £0.2 for DDT. Work was begun years ago to find a synthetic analogue but only recently have really significant advances been made. Since 1962, work on pyrethrin analogues has been carried out at Rothamsted supported by the National Research Development Corporation and two analogues, resmethrin and bioresmethrin, have recently been produced which are a considerable advance on the analogues previously available, notably tetramethrin and bioallethrin.

The new synthetic products have a kill rate many times higher than the natural product. Their knockdown rate is, however, lower although high when compared to other insecticides. This has been overcome by mixing them with the natural pyrethrin and a synergist which increases their effect.

The synthetic products appear to have all the advantages of the natural pyrethrins, without the drawback of the natural pyrethrins of causing nasal irritation if present in the air in sufficient quantities (pyrethrins are often used in aerosols). Indeed, the synthetic pyrethrins seem to be safer; the acute oral toxicity (LD₅₀, rat) of bioresmethrin is approximately 8,500 mg per kg of body weight, that of resmethrin approximately 2,000 and that of natural pyrethrin approximately 600. In feeding trials conducted by Cooper McDougal and Robertson, one of the two companies marketing bioresmethrin in Britain, no ill effects have so far appeared.

Where the potential of synthetic pyrethrins may lie is in agricultural applications where cost has so far prevented the use of natural pyrethrins. At present synthetic pyrethrins are even more expensive than the natural product (about £40 a kilo for resmethrin and

about £65 for bioresmethrin), but their relative non-toxicity, allied to their high kill rate, could make them a viable substitute for organochlorine and organophosphorus insecticides, many of which tend to break down less quickly than the pyrethroids.

The only known drawback to pyrethrin, both natural and synthetic, is that it is highly toxic to fish. This may not limit its use near rivers, however, as both water and light produce rapid breakdown, and tests may well prove that the quantities in which it will be used will present no danger to life in nearby water, should it be used for agricultural applications.

At present, however, the analogues are competitive with pyrethrum for a wide variety of largely domestic uses, and will be used overseas to replace other insecticides in household aerosols.

The development of synthetic pyrethrins has led to speculation that the market for the natural product may disappear, and Coopers admit that that was their first reaction, but the present need to mix synthetic with natural pyrethrins to achieve rapid knockdown, plus the expected expansion of the market, may well mean that the natural product is safe for some time to come. Kenya produces a large share of the world's natural pyrethrum and a spokesman at the Kenya High Commission said this week, "We think that synthetic pyrethrum will have some effect."

Ironically it was a world shortage of pyrethrum, caused by a fall of exports, that brought about the rapid commercial exploitation of the analogues, although Kenya claims that its output is now rising again. But whether the market for natural pyrethrum disappears—and experience with other synthetic substitutes for a natural product, for example, quinine, suggests it might—the future for the pyrethrum analogues looks bright.

COAL

Robens Recollects

LORD ROBENS's first public address since leaving the Coal Board surveyed the position of coal during this century and speculated on the uses of coal in future years. His address, the twentieth, and possibly the last, Coal Science Lecture of the now defunct Coal Utilization Research Association, was given on Monday to 400 scientists—most of them employed in coal research.

Lord Robens declared that more efficient use of man's resources must be made. In particular, it is now becoming clear that coal, which was considered the poor relation of gas and oil until very recently, has an important part to play in the future energy equation of Britain. Coal is Britain's only indigenous energy source and, although

the rich veins are being fully mined, a large supply of low quality coal is, as yet, unexploited.

One of the chief efforts of the coal board to this end has been directed towards the efficient burning of low grade coal and coal dust by fluidized combustion. In this process a mixture of coal and ash constitutes the fluid bed and the NCB effort has been devoted to obtaining a high efficiency of heat exchange between this and immersed boiler tubes. The practicability of such systems has been established in the laboratory and future effort will be devoted to making the system work on a large scale. A further advantage of the fluidized bed combustion is, as Lord Robens pointed out, its ability to suppress the emission of sulphur into the atmosphere when limestone is mixed with the fuel. This aspect of the process has interested scientists in the United States where there are large undeveloped supplies of low grade fuels and Lord Robens issued a warning that this might yet be another technique developed in Britain but exploited in the USA.

One of the problems that will face Britain towards the end of the century will be the gradual decrease in supply of North Sea gas. Lord Robens pointed out that efforts should be made to supplement this by synthesizing a gas that can be blended with supplies from the North Sea. At present the Gas Council is devoting considerable research effort to synthesizing gas from oil and Lord Robens stressed that in view of the uncertain future of oil—which is obtained mostly from politically unstable countries—efforts should be made to obtain such a gas from coal. Historically the way to achieve this has been by coking but this process is not efficient. Also the gas obtained has a low calorific value that is not commensurate with natural gas. The British effort towards achieving this end is at present concentrated solely in a combined Gas Council-NCB working party.

The NCB was, in Lord Robens's view, caught unawares in the early 1960s by the full implications of the Clean Air Act. The board was faced with having to develop in a short time either a smokeless solid fuel or appliances that would burn bituminous coal smokelessly. The board's quandary was finally resolved when it was decided to proceed with making smokeless fuels but Lord Robens stressed that this was no easy decision and, as he aptly put it, "the choice between the two approaches was by no means a simple black and white one." He added that it took ten years to solve all the technical problems of making smokeless fuels, after a smaller research effort into the design of appliances to burn bituminous coal smokelessly.

NEW WORLD

Fighting the Great ABM Battle Again

by our Washington Correspondent

A REPORT sharply critical of the professional conduct of some of the scientists who took part in the 1969 debate on the Safeguard anti-ballistic missile system has triggered off a controversy in the scientific community that could seriously damage relationships between scientists and Congress. Whatever the quality of the report and the validity of its conclusions (both of which are being vigorously contested), there is little doubt that it will be remembered for the impression it has generated—that prominent participants in the ABM debate sought to mislead Congress and the public on technical matters. Also at issue are the ethics involved when a professional society is seen to ally itself with participants on one side of a highly political debate by impeaching non-members for their alleged professional misconduct.

The basis of the controversy is a report written by an *ad hoc* committee of the Operations Research Society of America (ORSA) and published last week in the society's journal. Based on a study instigated at the request of Professor Albert Wohlstetter, the chief conclusion of the committee is that "when prominent experts outside the Administration supported their opinions on Safeguard deployment with arguments or results of an operations-research nature, these analyses were often inappropriate, misleading or factually in error".

The six-member committee also says that opponents of the Safeguard system were more unethical than their colleagues who supported the Administration. In particular, the report singled out for criticism Dr Jerome Weisner, Dr George Rathjens and Dr Steven Weinberg, all from Massachusetts Institute of Technology.

For their part, however, the MIT scientists charge the committee with lack of professional ethics because it took a narrow and biased view of the debate and failed to comment on the arguments and alleged mis-statements of supporters of the ABM. The MIT group also suggests that the analysis may have been instigated for personal rather than for altruistic reasons, and points out, in a rejoinder to the report, that whatever the findings of the *ad hoc* committee, arguments put forward by opponents of

the ABM have been vindicated by subsequent events.

The report concentrates chiefly on the arguments which surrounded the possible vulnerability of US Minutemen silos to pre-emptive attack by the Soviet SS-9 rockets, paying little attention to

the fierce debate over the ability of Safeguard to do the job specified for it and ignoring almost totally the discussions on whether or not the system could easily be overwhelmed or made useless by destruction of the Missile Site Radar (MSR) which guides the

THE Operations Research Society of America was launched into the ABM debate by a letter sent in November 1969 by Professor Albert Wohlstetter to Dr Tom Caywood, then president of the society. The letter listed points of difference between calculations performed by Wohlstetter and by opponents of the ABM, in particular by Rathjens, Weisner and Weinberg. Rathjens, Weisner and Weinberg responded to an invitation to cooperate by saying: "Any general inquiry that would widen the public's understanding of the issues would be welcomed by us but we feel that for the Operations Research Society of America to carry out an inquiry into the ABM debate along the lines suggested by Professor Wohlstetter would be absurd". The scope of the inquiry would be too narrow, the MIT scientists suggested, and "could well appear to the nation as an ugly resurgence of those attacks on civil liberties and dissent that were far too common fifteen years ago".

One member of the committee, Dr Howard Berger, a former president of the Military Operations Research Society, had had a serious disagreement with Rathjens when both were employed at the Institute of Defense Analysis. According to Rathjens, the disagreement led to his relieving Berger of responsibility for a project, and eventually to Berger's resignation from the institute. Caywood said last week that he was aware of some disagreement between Berger and Rathjens when he formed the committee.

The *ad hoc* committee presented its report to a meeting of the ORSA council in May this year, at which it was adopted by a vote of 11 to 1, with only Professor John Little of MIT dissenting. Subsequently, however, five members of the council submitted a minority statement for inclusion in the journal alongside the report, pointing out that they consider it inappropriate for an organization such as ORSA to take on the quasi-judicial function of investigating the professional behaviour of individuals. The ORSA council is therefore now itself divided on the issue of whether it should have carried out and published the report, and to add to the internal troubles of the organization, Professor Philip Morse, also of MIT and a founder member of ORSA, wrote a letter to the *Boston Globe* last week in which he strongly protested against the report and threatened to resign from the society. The burden of his argument is that, by denouncing by name individuals who are not members of the society, the report suggests ORSA is "on the side of ex-Senator Joe McCarthy, is promilitary and supports the assumption that experts always know best".

When the report was published last week, Rathjens, Weisner and Weinberg sent out a rejoinder in which they said that the report had substantiated virtually every criticism raised in their original letter to Caywood. The rejoinder was not, however, sent to members of the committee, or to Wohlstetter, who complained in an interview that this was simply another instance of the MIT scientists' "form of arrogance and elite contempt of the lay public".

Professor Little has taken the different view that, since the society should not have attempted an analysis of the debate and since it should not have placed itself in a quasi-judicial position, Rathjens had every right not to take part.

intercepting rockets to their targets. The committee says that it chose to study these areas because of their importance to the debate and because the calculations are simple enough to allow major points of difference in the assumptions underlying the various calculations to be singled out.

The ORSA report chooses to analyse in particular the arguments put forward by Rathjens and by Wohlstetter, which respectively came up with predictions that 75 and 95 per cent of the US Minutemen force could be destroyed in their silos by the stock of SS-9 rockets thought to be available to the Soviet Union by the mid-1970s. Rathjens argued on the basis of his calculations that the threat was not sufficient to justify deployment of Safeguard, while Wohlstetter suggested that if 95 per cent of the US retaliatory force of intercontinental ballistic missiles (ICBMs) could be knocked out by a pre-emptive strike, then they needed protecting.

The *ad hoc* committee found in effect that Wohlstetter's calculations were correct, and that he had presented them in a professional manner, but that Rathjens's performance was less than adequate. In particular, the report suggests that Rathjens assumed that the SS-9s would have less than their possible complement of independently targeted warheads (MIRVs), that he misread a chart presented in testimony to the Senate Armed Services Committee by Deputy Secretary of Defense David Packard, and that all the MIT scientists, together with Dr Wolfgang Panofsky, Director of the Stanford Linear Accelerator Center, ignored the possibility that some of the SS-9 rockets that may fail during launch or in flight could be replaced by other rockets by an intricate system of reprogramming. The committee finds that if all these alleged errors in calculation are taken into account, a pre-emptive strike by SS-9s could be expected to dispose of 95 per cent of the undefended Minuteman silos by 1976, if the trends in Soviet arms deployment evident in 1969 were maintained.

Rathjens assumed in his calculations that the SS-9s would each have four MIRVs, each with a yield of one megaton, while Wohlstetter based his calculations on the assumption that the missiles would each carry three five-megaton warheads. The basis of Rathjens's assumption is claimed to be data supplied by Deputy Secretary of Defense Paul Nitze in 1967, and he pointed out in a letter published in the *New York Times* in June 1969 that he assumed the SS-9 payload before the Administration suggested that each would have three five-megaton warheads. In any case, Rathjens points out that the payload likely to be employed on the SS-9 is not the most sensitive assumption in the calculation, and that he is not even now

convinced of the Soviet Union's ability to deploy large yield warheads on its missiles. Nevertheless, in the rejoinder to the ORSA report, Rathjens does not answer this particular criticism of his calculations.

As for the suggestion that he misread the chart submitted by Packard, Rathjens admits that he assumed the chart was calibrated in statute rather than in nautical miles and that this misreading led to his adoption of an unduly high value for the blast resistance of the Minuteman silos. But as he pointed out in a letter to Wohlstetter in June 1969, this error in fact was offset by an error in the accuracy of the SS-9, which also occurred from misreading the Packard chart in statute miles.

The chief point of disagreement between the opponents and proponents of Safeguard in 1969 was whether or not the SS-9s could be reprogrammed so that those which failed in launch or in flight could be replaced by fresh rockets.

The OSRA report suggests that if Rathjens's calculations are altered to include the correct value for blast resistance of the Minuteman silos and a substitution is made to include three five-megaton instead of four one-megaton warheads in the SS-9, the number of Minutemen assumed to survive a first strike is reduced from 25 per cent to 16 per cent. But if the attacking missiles can be reprogrammed to replace some failures, only five per cent of the Minuteman silos would remain intact.

Why did the opponents of the ABM not consider reprogramming? According to the MIT rejoinder to the ORSA report, they did not consider sophisticated reprogramming to be technically feasible, particularly reprogramming to compensate for failure of a single re-entry vehicle that had gone astray. The ORSA report points out, however, that failures occurring during launch and during early flight could be compensated for relatively easily with a significant improvement in the efficiency of the strike.

The rejoinder in fact does not attempt to rebut the ORSA report point by point. In fact, Weisner, Rathjens and Weinberg point out that they feel no obligation to do so but instead attack the report for not tackling all of the relevant issues. In particular, they suggest that the ORSA committee should have looked at the arguments concerned with the vulnerability to attack of the missile site radar, especially since the Administration is now admitting the limitations of the system and is looking into designs for an improved defence of missile silos, they chide the ORSA committee for not investigating the possibility that Safeguard may be overwhelmed, they point to a statement attributed to President Nixon that the Safeguard system could "provide a virtually infallible defence"

against Chinese attack, and ask why it was not examined, and finally they suggest that an examination of the shifting justification of Safeguard could well have found a place in the report.

There is little doubt that opponents of the ABM employed incorrect assumptions at times and came up with incorrect conclusions as a result (in fact the rejoinder admits as much), but Weisner and his colleagues point out that their calculations have been proved essentially correct by events. For example, they point out that the threat suggested by the Administration from the SS-9 has not materialized—the Administration was predicting a force of 420 SS-9 missiles by early 1972, but according to testimony given by Dr John Foster to the Senate Armed Services Committee on April 19 this year, the USSR has about 300 SS-9s and the rate of deployment was slowed down. A recent study carried out by TRW Inc., a California firm, suggests that the re-entry vehicles on the SS-9 may not be accurate enough to pose much of a threat to Minuteman silos, at least not a sufficient threat for a first strike capability—and the vulnerability of the missile site radar is now recognized and the Department of Defense is pressing ahead with design of a system specifically designed for protection of Minuteman (the Hardsite system).

Whatever the merits of the case that the committee has argued—and it cannot be denied that it has provided some evidence of errors—the report is likely to achieve little more than to cast doubt on the validity of testimony of future witnesses before Congressional committees. Moreover, in presenting what seems to be a one sided investigation, at least as far as examining and reporting on statements and arguments from both sides is concerned, the report has caused internal strife in the Operations Research Society of America itself (see box). In any case, there seems to be little point in digging over past debates when a more productive exercise would be to examine the validity of present assumptions about the ABM system.

CANCER

Defence Therapy

TERMINAL cancer patients at the University of Cincinnati College of Medicine are being exposed to large doses of radiation in a study paid for by the Department of Defense. One aim of the study is to determine the likely effect on troops of radiation from nuclear weapons, but members of the Cincinnati team claim that the treatment is given primarily for therapy and that it does not harm the patients. Since the

details of the study were disclosed last week in the *Washington Post*, however, they have raised some doubts about the ethics of undertaking this type of research.

Total body radiation therapy has been used at the University of Cincinnati for the past fifteen or sixteen years, according to Dr Edward Gall, one of the Cincinnati team, but the investigative part funded by the Department of Defense has a history of eleven years. Dr Gall claims that the Pentagon became interested in the radiation therapy when some of the results were first described, and although the programme has been expanded the therapy has not been altered. In effect, he claims that the Department of Defense is simply interested in results derived from normal therapy at the hospital.

The therapy involves exposing patients suffering from cancer that has progressed beyond surgery to total body radiation of between 100 and 300 rads, or occasionally to partial body doses of 50–100 rads. The Department of Defense is interested in monitoring the doses of radiation that produce nausea and vomiting and other effects that might impair the performance of troops on the battleground. Patients are told that the results of the treatment may be helpful in case people are exposed to large doses of radiation, but they are not told that the study is being funded by the Department of Defense.

So far, 81 people have been given the total radiation treatment at the University of Cincinnati, but 108 have been approached—27 were excluded either because they were found to be unsuitable for treatment or because they had indicated that they did not want to take part. They included patients suffering from cancer of the colon and lung and Ewing's tumour, and all had already been given treatment at the University of Cincinnati hospital. They are told that the therapy may relieve some of their pain but that it will not necessarily lengthen their lives. But they are not told that they may suffer nausea from the treatment, since that may bias the results.

The treatment is approved each year by the Committee on Human Research at the university. Some other cancer therapists have expressed doubts about both the ethics of the research and the efficacy of the therapy, however. In particular, it has been suggested that total body radiation is not used as a therapy elsewhere, and the Department of Defense says that it is not funding any similar research. The Cincinnati team, although unwilling to claim substantive evidence of success from its therapy, points out that out of four children treated for Ewing's tumour, three have survived for three and a half years.

DDT

Borlaug's Warning

by our Washington Correspondent

AN impassioned plea on behalf of DDT was delivered in Washington last week by Norman E. Borlaug, recipient of the 1970 Nobel Peace Prize for his work on high yield wheat strains. Borlaug, who testified in a public hearing on the cancellation order imposed on the pesticide by the Environmental Protection Agency, said at a press conference later:

"Environmentalists today seek a simple solution to very complex problems. The pollution of the environment is the result of every human activity as well as the whims of nature. It is a tragic error to believe that agricultural chemicals are a prime factor in the deterioration of our environment.

"The indiscriminate cancellation, suspension, or outright banning of such pesticides as DDT is a game of dominoes we will live to regret.

"DDT, because it is a name popularly known to most segments of the public, has been the first target. Once that is accomplished, the so-called ecologists will work on hydrocarbons, then organophosphates, carbamates, weed killers, and, perhaps, even fertilizers will come under the assault of their barrage of misinformation.

"If this happens—and I predict it will if most DDT uses are cancelled—I have wasted my life's work. I have dedicated myself to finding better methods of feeding the world's starving populations. Without DDT and other important agricultural chemicals, our goals are simply unattainable.

"Perhaps more than any other single factor in the world today DDT has a unique contribution to the relief of human suffering. I need not reiterate its vital importance in malaria control.

"DDT critics will say, of course, that only domestic uses of the chemical are being reviewed in the hearings at which I appeared today. But I have spent my life working in the nations of the world to help them feed themselves. I know how they will react if we terminate uses of DDT in this country and, in effect, label it 'poison'. If it is not good enough for your purposes, they will reason, then it shouldn't be used in our countries. The impact will be catastrophic."

DRUG ADDICTION

Fort Worth Deleted

by our Washington Correspondent

PLANS to transfer a drug addiction research and rehabilitation centre from the National Institute of Mental Health to the Bureau of Prisons seems to be going ahead in spite of stiff opposition from powerful members of Con-

gress. The centre, situated at Fort Worth in Texas, was due to be transferred to the Bureau of Prisons this month (see *Nature*, **232**, 597; 1971), but in July the House of Representatives blocked the move with a resolution designed to keep open all Public Health Service hospitals and outpatient clinics pending the findings of a thorough review of their functions. The resolution has, however, reportedly been emasculated in a conference committee appointed to iron out differences in the versions of the resolution passed by the House and by the Senate, and all references to the two research centres at Fort Worth and at Lexington in Kentucky have been deleted.

Opponents of the plan, led by Paul G. Rogers, chairman of the House Subcommittee on Public Health and Environment, argued that transfer of the Fort Worth centre to the Bureau of Prisons would in effect close down badly needed facilities for the treatment of drug addicts. NIMH officials have, however, countered that the loss of facilities will be offset by a sharp increase in the number of community health centres offering rehabilitation treatment and that, in any case, an addict is more likely to be rehabilitated if he is treated in his own community and not an isolated hospital.

While discussions about the future of the Fort Worth centre were taking place in Washington, however, many of the hospital's staff left, giving rise to serious doubts about the ability of the hospital to carry on operating in its present role, even if Congress did grant a stay of execution.

If, as now seems likely, the Fort Worth centre is transferred to the Bureau of Prisons for use as a medium security geriatric, alcohol and drug addiction hospital for prisoners, the move would underline the failure of the Narcotic Addict Rehabilitation Act of 1966. That act gave the courts the power to commit drug addicts for treatment instead of for prosecution for certain non-violent crimes such as mail theft, stealing cars and forging cheques.

Addicts committed under the terms of the Narcotic Addict Rehabilitation Act are sent to the Lexington and Fort Worth clinical research centres for detoxification and are then usually sent to aftercare centres in their local communities. In the first three full years of operation of the act, however, between July 1967 and June 1970, only 179 addicts were committed for treatment, and only 509 were committed after trial.

Congress had expected that about 900 addicts a year would be committed before trial alone, and that Lexington and Fort Worth would be bursting at the seams. Instead, the hospitals are running at half strength and seem destined for other things.

NEWS AND VIEWS

Seafloor Spreading and Mountain Building

CAN tectonic events on the continents be related to seafloor spreading several thousand kilometres away? The article by P. J. Coney on page 462 of this issue of *Nature* and that of R. W. R. Rutland in an earlier issue (*Nature*, **233**, 252; 1971) relate mountain building episodes in North and South America to changing seafloor spreading in the Atlantic. The bordering areas, the Caribbean and Scotia Arc, have also been studied recently in the light of seafloor movements by G. L. Freeland and R. S. Dietz (*Nature*, **232**, 20; 1971) and by I. W. D. Dalziel and D. H. Elliot (*Nature*, **233**, 246; 1971). This sudden flurry of articles reflects how details of seafloor spreading are now becoming available so that it is possible to relate such distant events with each other.

Perhaps the chief reason why seafloor spreading was not considered seriously until the 1960s was the apparent discrepancy between the essentially simple, localized pattern of spreading compared with the extensive, complicated patterns of mountain formation. The creation of new ocean floor takes place in an extremely narrow zone, possibly less than 30 m wide, at the centre of the ridge system, and this new oceanic lithosphere eventually descends back into the Earth's mantle at the oceanic trenches—again narrow zones compared with mountain systems of up to 1,000 km width. Between these two narrow active zones, the tectonic picture is quiescent; oceanic lithosphere is moving towards the trenches at different rates in different areas, although it is consistent over vast lithospheric plates on which only sedimentation is taking place, and the odd, persistent hot spot in the Earth's mantle is giving rise to a trail of volcanic islands. In contrast, mountain building occurs over wide regions of the continental crust with many phases of folding, faulting, igneous activity and metamorphism.

The discovery of major transform faults in the oceanic basins provided the first indisputable evidence of crustal movements of the magnitude required by the proponents of continental drift. On the whole, however, these faults could not be traced inland so that, for several years, it still seemed that there was little relation between the tectonics of the ocean floors and those of the continents. The

establishment of plate tectonics indicated that there must be at least some broad correlation between these tectonically active areas although it still seemed improbable that detailed correlations would be made between changes in spreading rate and direction of the plates and the precise sequence of events in an orogenic system, even on the same plate.

It is, however, the simplicity and narrowness of the mechanism by which new ocean floor is added that are allowing determinations of detailed movements of the plates. If the creation of seafloor took place over a wide region, many of these details would be lost in the "noise level" of the changing polarity of the Earth's magnetic field. These details, as they become available, are, however, becoming increasingly linked to specific changes in the degree of deformation, changes in the motion along faults, and so forth, within the orogenic systems of the continents. The current series of articles, such as that by Coney, mark only the start of this detailed correlation as they are based, so far, on magnetic records from those parts of the oceans which seem to reflect the simplest patterns, that is, where the locus of spreading has remained fixed, although the rate and direction of motion have changed. Coney is therefore able to distinguish three principal changes in spreading of the Atlantic plate from its initiation 150 to 180 million years ago, at 80, 40 and 9 million years ago. This means that he is able to correlate, for example, the evolution of the Azores with the Laramide orogeny and so forth, with only slight reference to movements of the Pacific plate.

Areas, such as the Atlantic north of Ireland, will take longer to interpret as changes have also taken place in the position of the axis of spreading. Similarly, the discovery that not all plate motions are symmetric with respect to the oceanic ridges (J. K. Weissel and D. E. Hayes, *Nature*, **231**, 518; 1971) must complicate the interpretation in some regions. Clearly, however, a synthesis of oceanic and orogenic motions on the Atlantic plates is becoming more feasible and, before long, the interaction between different plates will begin to emerge so that the tectonic activity will be seen in its true global perspective.

Putting the Cerebellum to Sleep?

ON page 481 of this issue of *Nature*, Siggins, Hoffer, Oliver and Bloom add another link to the growing chain of evidence which indicates that brain stem neurones rich in catecholamines are important regulators of activity levels in the cortices of the brain. They have demonstrated in rats that electrical stimulation in the neighbourhood of one such group of neurones leads to a prolonged inhibition of the impulse discharge from Purkinje cells—the output neurones of the cerebellar cortex. The amine-rich neurones concerned are those of the locus coeruleus (LC) in the rostral part of the pons. That axons of LC neurones terminate in the cerebellar cortex was first shown using histochemical techniques but the work of Siggins *et al.* represents the first attempt to use neuro-

physiological techniques to investigate the functioning of the pathway at the single neurone level. The potential importance of the path is emphasized by the finding that a single LC stimulus can produce a detectable decrease in the probability that individual Purkinje cells will discharge impulses; repetitive stimulation often leads to complete suppression of discharge.

It is difficult to speculate on the role of the projection in the normal operation of the cerebellar machinery. As Siggins *et al.* recognize, it has yet to be shown that the inhibition observed was directly mediated by synapses between the LC axons and the Purkinje cells. Such synapses have not been described but electron microscopical studies may yet help. At any event the long

latent period of the effect suggests that the path is unlikely to be important in that aspect of cerebellar function which currently attracts most attention—the control of evolving movements. A role as a long-term regulator of cerebellar cortical responsiveness seems more plausible.

Interest in the result is enhanced when it is viewed against a more general background. Histochemical studies by Olson and Fuxe (*Brain Res.*, **28**, 165; 1971) have shown that the axons of LC neurones terminate at destinations as diverse as the cerebral cortex, the hypothalamus and the lateral and medial geniculate bodies, not to mention the pathway terminating in the cerebellar cortex. The pathway to the cerebral cortex has been investigated in a general way by observing the behavioural changes caused by pharmacological agents believed to stimulate or to depress it. These experiments suggest that high LC activity leads to behavioural arousal and an alerting of the cerebral cortex and this activity is signalled by desynchronization of the electroencephalograph. Reduced activity in the nucleus, or outright destruction of it, leads to the synchronous electroencephalograph which is characteristic of light sleep or waking repose. The new results apparently show that the LC axons to the cerebellum are inhibitory and the odds are therefore high that the other LC projections are also inhibitory, in which case the path to the cerebral cortex clearly does not cause arousal directly by exciting cortical neurones. It may prove necessary to regard the projection as functioning by turning down some cerebral cortical element the unchecked activity of which leads to electroencephalographic synchronization and behavioural repose.

HAEMOGLOBIN

Hammering the Sickie

from our Molecular Biology Correspondent

THIS week, dear reader, a tale with a moral, about molecular remedies for molecular disease. Sickie-cell haemoglobin is in all respects a normal functional molecule, except for the property, which the single substitution in the β -chain confers on it, of associating in the deoxygenated state to large tactoidal aggregates. When this happens in the cell, the consequences are exceedingly disagreeable. A simple problem, one

might suppose, and an open challenge to protein chemists, to prove that theirs is a useful art, such as the hard-faced men by the National Institutes of Health should appreciate in tangible form. Better not to dwell on the more risible details, but to say only that until now they have failed prodigiously.

A new approach by a group of clinicians to the treatment of the condition was recently given wide publicity: having apparently encountered allusions to hydrophobic interactions between proteins, and the effect of urea on such forces, they proceeded to inject their patients with urea solutions. Protein chemists, knowing about the urea concentrations that might be effective in such situations, and guessing at the difficulties which might be involved in filling a patient with 8M urea, might have been permitted a pitying sneer. Astonishingly, however, this primitive ruse seemed to work. It is now apparent that this was in fact a consequence of the gradual formation in urea solutions—as every protein chemist also knows—of cyanate ions, which react with protein amino-groups.

The urea (which would not work anyway if too freshly made up) should therefore clearly be replaced by suitable doses of sodium cyanate. Kraus and Kraus (*Biochem. Biophys. Res. Commun.*, **44**, 1381; 1971) have now improved on this strategy by introducing carbamyl phosphate, a physiological substance, which is evidently capable of entering the red cell, with beneficial effects on the sickling tendency. They suggest that because phosphates in general compete with the natural cofactor, 2,3-diphosphoglycerate, for its site on the haemoglobin molecule, the carbamyl phosphate functions as an affinity reagent, entering the putative diphosphoglycerate site at the N-termini of the β -chain, and carbamylating the α -amino-groups (which indeed would be the most reactive at physiological pH). Indeed Caldwell, Nagel and Jaffé (*ibid.*, 1504) have shown that not only cyanate ions, but also carbon dioxide, which is known to bind non-covalently at the same site, will chase off diphosphoglycerate from normal human haemoglobin. One may also reflect that the loss by carbamylated haemoglobin of the capacity to bind the cofactor will lead to a relative increase in oxygen affinity (because the diphosphoglycerate binds only to the deoxygenated form). Thus the red cells will be less readily deoxygenated, and if they contain sickie-cell haemoglobin, the sickling will be inhibited. It is interesting to note that this provides a new retrospective rationale for the attempts once made to mitigate sickie-cell anaemia by administering carbonic anhydrase inhibitors. In addition to the decrease in pH that the accretion of

carbon dioxide will engender, thereby bringing into play the Bohr effect to increase the oxygen affinity of the haemoglobin, inhibition by carbon dioxide of diphosphoglycerate binding would work in the same sense.

The ramifications of diphosphoglycerate binding are evidently endless. Lian, Roth and Harkness (*ibid.*, 151) now report that in intact red cells, the shift in the oxygen uptake curve on addition of the cofactor is substantially increased when the cells contain sickie-cell haemoglobin. It has long been known that the oxygen affinity of such cells is lower than those containing only normal haemoglobin, and Lian *et al.* also now find that this difference is still present, though much diminished, when the cofactor is absent. These findings must be set against the well-established identity of both the oxygen and diphosphoglycerate affinity of normal and sickie-cell haemoglobins in dilute solution, out of the cell. The situation is complex, and Lian *et al.* indulge in some contorted efforts at explanation. The formation, however, of aggregates in sickie-cell haemoglobin only at high concentrations must stabilize the deoxygenated state, and it is therefore reasonable to suppose that this will decrease the oxygen affinity, and increase the diphosphoglycerate binding in the cell which in turn will enhance the change in oxygen affinity.

The aggregation reaction in sickie cells is complicated by possibilities of participation of other forms of haemoglobin that are commonly present, such as normal adult and foetal haemoglobin. The true complexity of the situation has now been revealed by Bookchin and Nagel (*J. Mol. Biol.*, **60**, 263; 1971), who use the device of introducing cyanmethaemoglobins, which have the oxyhaemoglobin conformation, but do not bind oxygen, into mixtures. Sickie-cell haemoglobin forms gels only in the deoxygenated state, but the normal adult pigment will co-aggregate with it both in the deoxy- and the cyanmet- (that is, oxy-) conformations. Foetal haemoglobin by contrast will enter the aggregates in the cyanmet- but not in the deoxy- state. In another abnormal variant (Harlem C), capable of aggregating when deoxygenated, the interactions with other forms and other conformations are quite different. The results are by no means easy to explain in detail, but the authors are able, starting from the notion, originally advanced by Pauling, that the aggregates are helical structures, to make some inferences about the existence of separate secondary interaction sites across successive turns of a tight helix of low pitch; these are envisaged as distinct from the primary association in which the sickie-cell haemoglobin molecule is regarded as bivalent.

MITOCHONDRIAL DNA

Arrested Replication?

from our Cell Biology Correspondent

IF the exquisite experiments—for once that is no exaggeration—reported by Kasamatsu, Robberson and Vinograd in the current issue of the *Proceedings of the US National Academy of Sciences* (68, 2252; 1971) are anything to go by, it seems likely that the enigma of the mechanism of DNA replication will be solved by studies of mitochondrial DNA. And on reflexion that claim is not perhaps as surprising as it may seem at first sight, for the mitochondrial chromosome is after all probably nothing more than a heavily pruned bacterial chromosome.

Vinograd's group, in their peerless manner, have found that in two strains of mouse L cells growing exponentially about half the mitochondrial DNA molecules (closed circular DNA duplexes with a molecular weight of about 10×10^6) contain a short segment of triple stranded DNA called a displacement or D loop. This curious structure, revealed in their electron micrographs with a clarity that denies dispute, consists of a short chain of DNA which is apparently base paired to one of the closed circular DNA strands such that a corresponding segment of the complementary closed circular DNA strand is displaced and loops out from the rest of the molecule. By heating mitochondrial chromosomes with these D loops to 90°C in 0.03 M NaCl, the short chain of DNA is released—it sediments at 7S in sucrose gradients—and concomitantly the two closed circular strands snap back together and the D loop is lost.

As Kasamatsu and her colleagues comment, the sedimentation properties of mitochondrial DNA with D loops suggest that the short displacing strand is about 3 per cent of the total length of the mitochondrial DNA molecule. On electron micrographs it measures 3.2 to 3.5 per cent the length of the complete molecule and assuming that the lower estimates of its length are the most accurate the displacing chain must be about 450 ± 80 nucleotides long. This chain hybridizes with the light but not the heavy strand of L cell mitochondrial DNA and it is therefore probably a short segment of the heavy strand. Furthermore, the position of the D loop, and therefore the sequence of the 450 base segment of the heavy strand, seems to be unique.

Vinograd and his colleagues reach this latter conclusion from ingenious argument and experiment. As Clayton, Davis and Vinograd have shown, circular dimeric mitochondrial DNA molecules in human cells consist of two monomeric molecules joined head to

tail. If this is also true of dimeric mouse L cell mitochondrial DNAs there should be two D loops per dimer and they should be diametrically opposed and separated by one genome length. Measurements of the position of D loops in electron micrographs of such dimeric molecules fulfil these expectations.

In short the properties of D loops eliminate the idea that they are intermediates in some DNA repair mechanisms, for repair would surely not be restricted to a unique site in the genome. But because they are present in up to 50 per cent of the mitochondrial DNA molecules isolated from exponentially growing cells, D loops may be an early stage in the synthesis of progeny mitochondrial heavy strands during a replication process initiated at a unique origin. Vinograd and his colleagues suggest that the initiation and synthesis of the progeny heavy strand can occur

without a nick being introduced into either of the closed circular parental strands but growth of this chain is arrested when it is about 450 ± 80 bases long. Further extension is then dependent upon nicks being introduced into the parental strands to allow their free rotation and unwinding. The occurrence of molecules which have nicked parental strands and displacement loops which range in size up to that of the complete genome supports this interpretation. In brief, closed circular molecules with D loops may well be mitochondrial genomes which have begun their replication but are hung up awaiting the attention of a nuclease without which they cannot continue. No doubt we shall not have long to wait before Vinograd's group can tell us more about this process and also about the synthesis of progeny light mitochondrial DNA strands complementary to the new heavy displacing strand.

Geometric Refinement of Crystal Structures

ALTHOUGH known interatomic distances are often used to find a chemically acceptable crystal structure concordant with an observed diffraction pattern, they are hardly ever used in the subsequent refinement stages of the analysis. In next Monday's *Nature Physical Science* W. H. Baur shows how, in some contexts, they can be incorporated most profitably and even used to overcome shortcomings in the observed crystallographic data.

Least-squares refinement, the most widely used refinement procedure at present, attempts by adjustments of the atomic coordinates and thermal parameters to improve the agreement between the observed and calculated structure amplitudes. It relies on the fact that the number of observed structure amplitudes greatly exceeds the number of adjustable atom parameters, but the ultimate accuracy achieved for any structure depends on the accuracy and extent of the intensity data. In the past, geometrical constraints have only been introduced into the process to treat certain atoms as a rigid group.

Inorganic crystallographers, especially those concerned with silicates, enjoy a situation not shared by many of their colleagues, and this has been exploited by Baur. Inorganic crystals are based on dense packing of anions interspersed by smaller cations and, although the cation angles may be distorted, the wealth of interatomic data now available (much of it derived from accurate single-crystal studies) has shown that contact distances are remarkably uniform. In 1969 Meier and Villiger (*Z. Krist.*, 129, 411; 1969) realized that the

number of interatomic distances in these structures exceeds the number of adjustable atom coordinates, described a least-squares procedure for adjusting the coordinates to minimize the spread of distances of a given type and demonstrated the usefulness and scope of the procedure. Baur, who has elsewhere been able to show that changes of interatomic distance are linearly related to changes of bond strength (using an extended electrostatic valence theory) and to derive empirical constants for a number of atom pairs, has now combined these linear relations with Meier and Villiger's least-squares refinement of distances. In particular he has shown how a rather poorly defined structure for the medium-pressure β -polymorph of MgSiO_4 , originally deduced from powder data, could be refined to produce a far more homogeneous set of distances corresponding closely ($\langle \Delta d \rangle = 0.027$ Å) with those recently derived from a careful single-crystal study of the isomorphous β - CoSiO_4 . Experience gained with earlier trials had shown that even more preferential weight should be given to the cation-oxygen distances than had been given by Meier and Villiger, and that this weighting is critical. When applied to accurately known structures the approach has proved capable of predicting Si-O distances to 0.01 Å or less.

It is a pity that the method is not more widely applicable: those people who study molecular crystals will surely be forgiven if they seem a little envious of a technique that allows their colleagues to transcend the limitations of their data.

ELECTRON MICROSCOPY

High Voltage at NPL

from a Correspondent

THE six AEI 1 MeV electron microscopes (installed at UKAEA, Harwell, the National Physical Laboratory, Imperial College, the Universities of Oxford and Birmingham and the British Iron and Steel Research Association, Sheffield) plus the 1 MeV JEOL machine at the Central Electricity Generating Board, Berkeley, and the original 750 keV instrument in Cambridge represent a UK investment in high voltage electron microscopy far exceeding that of any other country. About eighty people—mostly materials scientists using these various instruments and a few biologists—attended a conference organized by the Institute of Physics and the Royal Microscopical Society in conjunction with the National Physical Laboratory at Teddington from September 22 to 23.

Dr A. Howie (University of Cambridge) reviewed recent progress in understanding the penetration of electron beams in crystals and the remarkable channelling effects occurring above the "critical voltage". This voltage and related interference fringe spacings can be accurately measured to give information about lattice potentials as was shown for gold by Dr J. Steeds (University of Bristol) and for uranium by Drs B. Hudson and A. J. E. Foreman (UKAEA, Harwell). Local temperatures or alloy composition and order can also be determined in this way. Dr E. Butler (Imperial College, London) showed that in nickel-gold alloys 1 per cent compositional change could be detected over distances of about 1μ . Use of a mini lens to reduce the size of the illuminating spot to perhaps 0.1μ could make this a very powerful microanalysis technique. The discussion indicated that an improved theory which takes account of non-systematic reflexions would be useful for this work.

Above the threshold energy at which the fast electrons can knock atoms out of their lattice sites, the high voltage microscope is unrivalled for radiation damage studies *in situ*. Void growth, which causes serious swelling problems in fast reactor materials, can be simulated in the microscope at a rate 10^4 faster than in the reactor (Dr K. R. Williams *et al.*, CEBG). Accurate knowledge of flux density in the beam and temperature rise in the specimen are essential for quantitative interpretation of these experiments, as was pointed out by Dr M. J. Makin (UKAEA, Harwell).

The transparency of crystals several microns thick to fast electrons allows *in situ* experiments which are reasonably typical of bulk materials. Results presented included recrystallization processes in copper alloys (Dr R. Ray *et al.*, University of Birmingham), growth of

strain-induced martensite in a titanium-zirconium alloy (Drs H. Flower and P. Swann, Imperial College, London) and an interesting film of dislocation motion during creep of polycrystalline aluminium (Drs K. F. Hale and M. Henderson Brown, NPL). The possible role of point defects in these experiments when the damage threshold energy is exceeded has not yet been assessed.

High voltage microscopes usually have more available space near the specimen which, in addition to the greater penetration of the electron beam, makes them very suitable for *in situ* experiments involving special stages. Some of these experiments were surveyed by Professor U. Valdré (University of Bologna) and several new straining stages and environmental cells were described. The latter have been used in studies of cement hydration (Dr D. Allinson, NPL), the reduction of haematite (Drs N. Tighe and P. Swann, Imperial College, London) and the observation of 70 Å fringes in wet, partially stained catalase (Drs P. R. Ward and R. F. Mitchell, University of Cambridge).

Dr A. Glauert (University of Cambridge) listed the chief advantages of the high voltage microscope in biology as the ability to observe wet (but not living) specimens and to obtain both better resolution and, with stereo methods, a better three-dimensional picture of the structure in thick specimens. The ionization damage in organic materials is re-

duced at higher voltages but, as the polymer work of Drs M. J. Richardson and K. Thomas (NPL) demonstrated, this is often cancelled by the increased exposure time required with the photographic plates currently used.

Professor J. M. Cowley (Arizona State University) described a 1 MeV transmission scanning microscope now under construction and emphasized the advantages (chiefly reduced chromatic aberration and improved signal processing) which this technique has over conventional microscopy. This scanning instrument, which operates below the damage threshold, may well (at the cost of some resolution) offer significant advantages for the transmission studies of thick crystals. Its role at higher voltages, whether or not the probable damage produced is of interest, is rather more uncertain and Professor Cowley's results will be awaited with interest. In the meantime, the bold initiative which placed the high voltage conventional microscopes at the disposal of workers in Britain is being followed up with imagination and vigour.

HEPATITIS

Among Drug Addicts

from our Medical Virology Correspondent
TRANSMISSION of viral hepatitis through the communal use of syringes and needles by addicts for the injection of

Messenger RNA and the Endoplasmic Reticulum

THE elucidation of the way in which messenger RNAs, transcribed in the nucleus of eukaryotic cells, are matured, packaged and transported to the cytoplasmic translation machinery is one of the great challenges of cell biology. Over the past several years evidence has been accumulated which suggests that messenger RNAs occur in cell nuclei in the form of ribonucleoprotein particles—so-called informosomes—and in *Nature New Biology* next week Faiferman, Cornudella and Pogo report experiments with Krebs ascites cells which, they believe, indicate that these ribonucleoprotein particles migrate to the cytoplasm and become associated with the endoplasmic reticulum.

Faiferman and his colleagues gently lysed Krebs cells which had been labelled with RNA precursors and, by differential centrifugation, isolated the cell nuclei to which remained attached most of the cell's endoplasmic reticulum. After disrupting the nuclei in a French press, the nuclear chromatin was pelleted and the supernatant was found to contain 40–50S ribonucleoprotein particles as well as fragmented reticulum. The 40–50S particles are

made in the presence of low doses of actinomycin D which block ribosomal RNA synthesis but cordycepin, which blocks messenger RNA synthesis in HeLa cells, blocks the synthesis of these particles in Krebs cells. To eliminate the possibility that the 40–50S particles are of mitochondrial origin, Faiferman and his colleagues have shown that their synthesis is not affected by ethidium bromide, which specifically inhibits the transcription of mitochondrial DNA.

Pulse chase experiments reveal that in the presence of amino-acids the 40–50S particles are rapidly chased into the endoplasmic reticulum. By contrast, when the cells are starved of amino-acids not only are the 40–50S particles broken down in the cytoplasm but also large numbers of ribosomes, which are normally attached to the reticulum, are found free in the cytoplasm. Faiferman *et al.* suggest therefore that the attachment of ribosomes and the 40–50S particles containing messenger are concurrent events. In short, they believe that messenger RNA is delivered to the reticulum in a 40–50S particulate riboprotein complex.

heroin, methylamphetamine and other drugs is a common and an increasingly serious problem in major urban centres throughout the world. The infection is spread by needles and syringes contaminated with blood. In a report from the Centre for Disease Control, Atlanta (*Hepatitis Surveillance Report*, No. 32; 1970), males between the ages of 15 and 29 years were identified as a high risk population for abusing parenteral drugs and the incidence of hepatitis among this age group is high. Once hepatitis is introduced into a group of drug addicts who use communal intravenous equipment, the disease spreads rapidly.

Cherubin and his associates (*Amer. J. Epidemiol.*, **95**, 510; 1970) detected the Australia antigen, which is specifically associated with serum hepatitis, in 54 per cent of patients who were known addicts and who were admitted with viral hepatitis to two hospitals in New York. In another group of young adults with clinical hepatitis who denied the use of drugs, Australia antigen was found in 50 per cent. On the other hand, the frequency of detectable antigen among healthy volunteer blood donors in New York is only 0.1 per cent. It was suggested that the high incidence of serum hepatitis in New York City might be attributable to a greater drug addiction problem than had hitherto been considered. It is, of course, true that the problem of hepatitis among drug addicts may well have been underestimated because there is some evidence that the virus of serum hepatitis may be transmitted not only by the parenteral route but also by the oral route and by sexual intercourse.

Another disturbing report has recently been published by Collins and Wells (*Milit. Med.*, **136**, 572; 1971) who noted a dramatic increase in the number of patients admitted with acute viral hepatitis to Brooke General Army Hospital between July 1969 and June 1970. The greatest increase in admissions was related to hepatitis associated with illicit drug abuse.

Drug abuse may be regarded in practice as a contagious disease because the habit is transmitted from one person to another. This approach makes it possible to apply to the study of drug addiction the methods used in the epidemiology of infectious disease and notification of hepatitis in young adults may well be used as one marker of drug abuse. Knowledge of the epidemiology of viral hepatitis among drug addicts is so far incomplete, but there can be no doubt that illicit drug users constitute an important reservoir of serum hepatitis in the community and the spread of infection to close family contacts, medical and nursing personnel and indeed to the population at large presents a real hazard.

ACETYLCHOLINE

Regulation of Binding

from our Neurochemistry Correspondent
A FURTHER addition to recent rapid advances in the application of techniques of molecular biology to the study of drug-receptor interactions (see, for instance, *Nature*, **229**, 523; 1971; and **231**, 91; 1971) is provided by evidence reported by M. E. Eldefrawi and R. D. O'Brien of Cornell University (*Proc. US Nat. Acad. Sci.*, **68**, 2006; 1971) that the binding of acetylcholine (ACh) to a preparation from the *Torpedo* electric organ containing cholinergic receptors is reduced at high concentrations of ACh.

Binding was measured by equilibrium dialysis using ^3H -ACh, and a particulate fraction in which all the acetylcholinesterase (AChE) was inhibited irrever-

sibly. The binding sites studied were probably acetylcholine receptors, because O'Brien and his colleagues have shown previously that the binding of muscarone (a stable cholinergic agonist) to the same preparation was reduced after treatment with a variety of drugs known to act at cholinergic receptors, but was unaffected by other drugs. In the present experiments, binding of acetylcholine was found to reach saturation at a concentration just below $1\ \mu\text{M}$, but was markedly reduced at higher concentrations.

As pointed out by Eldefrawi and O'Brien in their discussion, this "auto-inhibition" of acetylcholine binding to receptors is analogous to the reduction in activity of many enzymes, including acetylcholinesterase, at high concentrations of substrate. The latter effect,

Anti- θ Antisera and T Cells

In recent years cellular immunologists have come to rely heavily on means of identifying the cell populations involved in immune responses. It is no longer good enough to say that changes have occurred in lymphoid organs after an antigenic stimulus, though the classic approach of the histopathologist is still obviously a prerequisite in any comprehensive study. The means adopted for cell identification have been many and various and are in most instances still only suitable for experimentation with small rodents of which the genetic constitution in relation particularly to tissue strain and species specific antigenicity can more readily be controlled.

One of the most useful devices has been the use of the theta (θ) alloantigen as an indicator for (T) cells of thymic origin. A recent review by Raff (*Transplant Rev.*, **6**, 52; 1971) summarizes such work. The problems with θ , however, are numerous. It is not absolutely specific for cells of thymic origin because it has been known for some time that some brain cells are θ positive. This is usually a minor problem for the experimentalist, but more serious are the possibilities, first, that not all T cells have easily detectable amounts of θ , as has been suggested by Miller and Sprent (*Nature New Biology*, **230**, 267; 1971), and, second, that the anti- θ antisera in use are not completely specific for T cells, which is the burden of a paper by Greaves and Raff which is to be published in next Wednesday's *Nature New Biology*.

Greaves and Raff prepared two batches of anti- θ antisera by injection of CBA thymocytes into AKR mice. Both sera had the same cytotoxic titre in the presence of guinea-pig complement as adjudged by either dye exclusion or release of ^{51}Cr from labelled target cells.

It was found, however, that when the first serum (anti- θ_1) was used against lymphoid target cells in the presence of rabbit complement many more cells were killed than in the presence of guinea-pig complement. The conclusion was that a supposedly specific anti-theta serum was killing B cells. It is argued that the θ_1 antiserum contained an antibody directed against a previously unknown surface alloantigen which is present on the surface of B cells. The new antibody, however, required rabbit complement for expression of its cytotoxic potential.

Greaves and Raff present further studies in which anti- θ antisera were used to inhibit splenic rosette forming cells in the presence of guinea-pig complement. Anti- θ_1 had the capacity to inhibit both background and "immune" rosettes, but if it was absorbed with (autologous) AKR thymocytes its capacity to inhibit immune rosettes in the early phase of the response was limited. The authors conclude that anti- θ_1 contains an autoantibody which can act synergistically with anti- θ . Anti- θ_2 , their second batch of antiserum, was deficient in the autoantibody. A corollary is that reacting T cells are deficient in θ antigen, a conclusion in line with that of Miller and Sprent.

Thus it seems that care must be taken in the use of anti- θ antisera as absolute criteria of T cells. This may restrict the use of fluorescent anti- θ antisera on tissue sections—because of staining of non-T lymphocytes. It should not, however, be thought that the analyses of the composition of various lymphoid cell populations in terms of T and B cells by the use of anti- θ sera in the presence of guinea-pig complement are invalid. Such a conclusion would be premature and almost certainly wrong.

however, is usually caused by an alteration in the effectiveness of the catalytic process rather than of the binding.

Of greater interest is the possible relation between this autoinhibitory effect and the blockade observed physiologically when neuromuscular preparations are exposed to high concentrations of acetylcholine—the phenomenon of “desensitization”. Classical drug-receptor theory supposes that drug effects are a consequence of a change in the receptor to an “active” form, induced by binding of the agonist. On this basis, desensitization has been interpreted as being caused by the formation of “inactive” acetylcholine-receptor complexes, with a reduced ability to generate a physiological response. Receptors can thus exist in two forms, effective—R, at low ACh concentrations of acetylcholine—and desensitized—R', at high concentrations of acetylcholine. The model also assumes that the affinity of R' for acetylcholine is lower than that of R, but suggests no explanation of how these changes are brought about.

Eldefrawi and O'Brien propose a scheme whereby receptors may possess more than one type of binding site, occupation of low affinity sites leading to a reduction in binding to the high affinity or active sites and ultimately to a conformational change in the receptor, such that the affinity of the active sites for acetylcholine is decreased greatly. This would provide an attractive explanation for the molecular mechanism underlying desensitization, essentially analogous to the model for allosteric properties of enzymes. But so far there is no direct proof of multiple binding sites on receptors, and the authors should also perhaps first demonstrate a direct coincidence between physiologically observed desensitization and inhibition of binding *in vitro*.

BEHAVIOUR

Ethology in Edinburgh

from our Animal Behaviour Correspondent

THE wide range of topics discussed at the twelfth international ethological conference held in Edinburgh from September 7 to 15 reflects the diversity of interests of ethologists. The first morning of the conference was devoted to the neurophysiological mechanisms underlying sequences of invertebrate behaviour, and several speakers, including Dr A. O. D. Willows (Washington University), showed that patterns of overt behaviour may in some cases arise from patterns of impulses originating in particular neurones. Other plenary sessions covered imprinting, behaviour of social insects and orientation. Dr W. T. Keeton (Cornell University) reviewed the most recent evidence on the

homing ability of pigeons and concluded that biologists simply do not understand how these birds are able to find their way home. Displacement activities (behaviours appearing “out of context” or at least where ethologists don't expect to find them) were discussed by Dr P. Sevenster (Leiden University) and Dr D. J. MacFarland (University of Oxford).

Professor N. Tinbergen (University of Oxford) stressed the importance of investigating not only the causation of behaviour but also its adaptedness—how it helps the animal to survive—and Professor K. D. Roeder (Tufts University) pointed out that this applies as much to physiology (“behaviour below the skin”) as to ethology. As an illustration of the interaction between these two disciplines, he described the violent escape response of a sphingid moth to ultrasound, a response which he has recently found to depend on a novel type of sound receptor near the moth's mouth.

Dr R. P. Michael (Institute of Psychiatry) described work carried out at the Primate Research Centre at Beckenham which has revealed that the smell of certain substances in the vaginal secretions of female rhesus monkeys has

the effect of dramatically increasing sexual activity in the males. Dr Michael is continuing this work on other species including humans.

For the first time, the conference included a representative from the Soviet Union. Dr D. K. Belyaev (Novosibirsk University) described the unexpected morphological and chromosomal changes that took place in a population of silver foxes selected primarily for behavioural traits.

During the afternoons three parallel sessions were held in different halls so that it was impossible for everybody to hear all of the hundred or so contributions. Judicious flitting from one room to another, however, did enable one to learn of the ability of electric fish to recognize the sex of other individuals on the basis of the frequency of their electrical discharge (Dr C. D. Hopkins, Rockefeller University), of the precision with which caddis fly larvae build their houses (Dr M. H. Hansell, University of Glasgow) and of the function of the “dance” that blowflies perform when they come across a drop of food (Dr M. C. Nelson, Tufts University), to mention just three from several noteworthy contributions.

Reactivity of Portland Cement Minerals

OF the two calcium silicates, which form the bulk of Portland cement, $3\text{CaO} \cdot \text{SiO}_2$ is much more reactive than $\beta 2\text{CaO} \cdot \text{SiO}_2$. Both these phases, however, develop similar ultimate strengths on hydration and, from the manufacturing point of view, cement richer in $\beta 2\text{CaO} \cdot \text{SiO}_2$ is easier and cheaper to make than a cement richer in $3\text{CaO} \cdot \text{SiO}_2$. Various empirical attempts have thus been made to increase the reactivity of $\beta 2\text{CaO} \cdot \text{SiO}_2$ without gaining any fundamental understanding of the factors controlling the intrinsic reactivity of the minerals. In a communication to be published next Monday in *Nature Physical Science*, Ramachandran and Sereda have applied the Hedvall effect to gain some understanding of this difference in reactivities of $\beta 2\text{CaO} \cdot \text{SiO}_2$ and $3\text{CaO} \cdot \text{SiO}_2$.

AgNO_3 has a phase transition point at about 190°C . When a mixture of AgNO_3 and a reactive material, for example CaO , is cycled through this transition temperature a reciprocal pair forms very quickly. The authors have taken advantage of this fact in determining the reactivities of different minerals that may be present in Portland cement. The authors heated mixtures of AgNO_3 and an individual cement mineral (or fully hydrated $3\text{CaO} \cdot \text{SiO}_2$) in a DTA (differential thermal analysis) apparatus and esti-

mated the amounts of AgNO_3 used up to form reciprocal pairs in each case. From these data they calculated the quantities of Ca ions that took part in each case. For CaO and $\text{Ca}(\text{OH})_2$, all the Ca ions took part in the reaction, but in the case of $3\text{CaO} \cdot \text{SiO}_2$ and of $\beta 2\text{CaO} \cdot \text{SiO}_2$ only 27 per cent and 6 per cent respectively of the Ca ions were involved. The presence of a higher proportion of more reactive Ca ions in $3\text{CaO} \cdot \text{SiO}_2$ than in $\beta 2\text{CaO} \cdot \text{SiO}_2$ seems to account for the higher reactivity of $3\text{CaO} \cdot \text{SiO}_2$. The authors then infer that “in tricalcium silicate all Ca ions are not equivalent, a part of it being more reactive”.

The origin of this high reactivity of some of the Ca ions in tricalcium silicate is not so obvious. In the structure of $3\text{CaO} \cdot \text{SiO}_2$ (Jeffery, *Acta Cryst.*, **5**, 26; 1952), all the Ca ions are surrounded by six oxygen ions. In $\beta 2\text{CaO} \cdot \text{SiO}_2$ (Midgley, *Acta Cryst.*, **5**, 307; 1952) half of the Ca ions have six oxygen ions as first neighbour and the other half have eight. Thus origins of the higher reactivities of some of the Ca ions in different calcium silicates seem to be extra-structural, and they may not be directly related to the intrinsic reactivities of different calcium silicates. The origin of the difference in reactivities of the cement compounds thus still remains unexplained.

PALAEOLOGY

Mammalian Evolution

from our Vertebrate Palaeontology
Correspondent

THE nineteenth symposium on vertebrate palaeontology and comparative anatomy was held at Bristol on September 28–29, followed by two days of field excursions. The evolution of mammals, especially those of Africa, was a principal theme, because there is a large and active group at Bristol concerned with this subject. New work on the earliest known mammals, *Eozostrodon*/*Morganucodon* and *Kuehneotherium*, of the Triassic was described by Professor A. W. Crompton (Harvard University). Some workers have suggested that each of these two mammals gave rise to some of the groups of Jurassic mammals, but that they are so different from one another that they must have arisen from different lines of advanced mammal-like reptile—so making the mammals diphyletic. Professor Crompton strongly dissented from this view, pointing out a number of similarities between the two Triassic genera. He also noted that the epipterygoid of such cynodonts as *Probainognathus* is not greatly enlarged, indicating that this group could be ancestral to all the mammals, including the triconodonts (whose epipterygoid, unlike that of most other mammals, is small). An interesting discovery of early mammals in Britain was that of Dr M. Waldman (Stowe School). Though difficult to detect, and also difficult to remove from the hard matrix, his find of Middle Jurassic mammals in the Isle of Skye is of great potential interest, because the only other mammalian fauna of this age comes from the Stonesfield Slate of Oxford, which is no longer quarried.

Professor P. M. Butler (Royal Holloway College, London) suggested that the directions of jaw movement, as indicated by wear striations on the teeth, had passed through three principal phases in mammalian evolution. In Jurassic forms, the movement is partly transverse and becomes progressively more so, but the teeth shear past one another. Not until the Cretaceous do the teeth collide at the end of the jaw movement, so giving a crushing action. This in turn provided the possibility of a subsequent movement in another direction, so that two different sets of facets can be distinguished in the Tertiary herbivores and omnivores.

The studies of African mammals ranged from the problems of the phylogeny of the older forms to those of evolutionary rates and the comparison of later forms with those still extant. Mr G. J. Heal (University of Bristol) described sirenians which showed that two families of this order were already distinguishable in the Eocene, and Mr

A. W. R. Wight (University of Bristol) described new specimens of Proboscidea and discussed the Eocene stratigraphy of Libya. Dr V. Maglio (Princeton University) analysed the rates of appearance and replacement of species in the Elephantinae and suggested that the group was in the middle of a phase of adaptive radiation when, in the Upper Pleistocene, there was a dramatic crash from eleven lineages to the present two. Dr A. W. Gentry (British Museum (Natural History)) compared the African Pleistocene antelopes with those of the continent today and pointed out that, because horn shape is a species recognition feature, the whole antelope fauna of any one time, within which such distinctions were essential, should be considered in any analysis.

At the other end of the chordate evolutionary story, Dr R. P. S. Jefferies (British Museum (Natural History)) described a new mitrate echinoderm which he believed showed evidence of being ancestral to the cephalochordates. Professor D. L. Dineley (University of

Bristol) gave an interesting account of the Late Devonian fish fauna of Escuminac Bay, Canada, and Mr D. S. Broad (University of Bristol) described new heterostracans from Arctic Canada which showed evidence of the growth of each plate by accretion around a small number of original tubercles. The problem of interpreting the patterns of tetrapod tooth replacement was discussed by Dr R. DeMar (Liberal Arts College, Chicago), who showed that Edmund's Zahnreihe is only one of a number of theoretically possible alternatives, and that computer studies can show why some of these are not acceptable, because they lead to lengthy gaps in the tooth row.

Dr A. C. Charig (British Museum (Natural History)) pointed out that reptiles colonized more new ways of life in the Triassic than ever before or since, and suggested that this might be more than mere coincidence—though other possible reasons were not obvious. A valuable approach to the study of pterosaurs was presented by Dr G. R.

Visualizing Agglutinin Sites on Cell Surfaces

DURING the past several months the suggestion that transformed cells are more readily agglutinated by plant agglutinins than their untransformed counterparts because transformation leads to an increase in the number of agglutinin-binding sites exposed on the cell surface has been abandoned. For experiments such as those reported by Cline and Livingston and Ozanne and Sambrook in *Nature New Biology* (232, 155, 156; 1971) have shown that there is apparently no significant difference between the numbers of these binding sites on the surfaces of transformed and untransformed cells. These workers have suggested therefore that it is the distribution of agglutinin-binding sites rather than their numbers which is changed by transformation and in next Wednesday's *Nature New Biology*, Nicholson reports observing in the electron microscope just such a change; after transformation by SV40 virus the concanavalin A binding sites on the surface of mouse 3T3 cells are clustered whereas before transformation they are dispersed.

Nicholson was able to make these most elegant observations by preparing ferritin conjugated concanavalin A and then devising a new technique for preparing the cell surface membrane for electron microscopy. His technique involves first "strengthening" the cell membrane by briefly incubating the cells in 0.1 per cent formaldehyde before lysing them on an air–water interface and then picking up the membrane on electron microscope grids. Once on the

grids the membranes can then be stained with the ferritin tagged concanavalin A.

The micrographs of stained membranes from transformed and untransformed 3T3 cells reveal at a glance the striking difference in distribution of binding sites. Single ferritin tagged concanavalin A molecules are randomly distributed on the membranes of the untransformed cells. By contrast the tagged concanavalin A molecules are in clusters, which are randomly distributed, on the membranes of the cells transformed by SV40. Although there are some 3 to 3.5 times more molecules of bound concanavalin A per unit area of transformed than per unit area of untransformed cell membrane, Nicholson points out that the total surface area of the transformed cells is only about half that of their untransformed counterparts.

Nicholson has also shown that after exposure to weak trypsin solutions, the concanavalin A sites on the surface of untransformed cells tend to be clustered, but within a few hours of enzyme treatment the sites assume their normal distribution.

There could therefore scarcely be a clearer demonstration of the fact that transformation causes a change in the distribution of these agglutinin-binding sites, which presumably accounts for the increased susceptibility of these transformed cells to agglutination. Whether this particular surface change also alters the social behaviour of cells remains to be seen.

Whitfield (University of Reading), who outlined the way in which theoretical aerodynamic studies and conventional anatomical studies can combine to throw new light on the functional anatomy of *Pteranodon*.

In two studies on modern reptiles, Dr G. L. Underwood (City of London Polytechnic) explained a dendritic classification which provides a very satisfactory picture of the evolution of boid snakes, and Mrs L. Sheppard (St Mary's Hospital, London) showed that the joints between the neural arches in lizards and snakes contain menisci, and suggested that these provide a flexible and compressible element needed in joints which permit movement in more than one plane.

SCHIZOPHRENIA

Biochemical Aspects

from a Correspondent

THE first joint meeting of the schizophrenia associations of Canada, America and Great Britain was held in London on September 28 and 29. Work on the genetic basis of schizophrenia was reviewed by Mr J. Shields (MRC Psychiatric Research Unit, Maudsley Hospital, London) in relation to current hypotheses and he concluded that the evidence for the existence of genetic factors rests on general arguments. Dr L. R. Mosher (National Institute of Mental Health, Bethesda), referring to a study of identical twins discordant for schizophrenia, reported having found different patterns of interaction between one parent and one twin (who was later to become schizophrenic). The twin as a result was repeatedly less favoured and a relationship was established with a contradictory and incomplete role expectation and without cognitive and communicative clarity.

Schizophrenia is a changing concept, said Dr D. Richter (MRC Neuropsychiatry Unit, Carshalton); it has altered as various subgroups have been isolated from the chief group of schizophrenias. Dr A. P. Ridges (University of Liverpool) emphasized the need for a definition of the criteria used in selecting patients and conducting experiments under carefully controlled conditions. She supported Dr Richter and said that altered activity of several enzymes or more probably isoenzymes could finally affect common metabolic pathways producing the symptoms of schizophrenia. Pink spot excretion seems to be associated with an altered catecholamine metabolism in schizophrenia. She reviewed other biochemical considerations including the suggested association between coeliac disease and schizophrenia.

The possible central effects in schizophrenia were considered by Dr A.

Randrup (St Hans Hospital, Roskilde), who presented evidence indicating an association between schizophrenia and dopaminergic hyperactivity in the brain. Professor J. Yaryura-Tobias (University of Buenos Aires) considered that the basal ganglia are of prime importance in the aetiology of schizophrenia. Dr K. Smith (Washington University) reported that she has found trans-3-methyl-2-hexenoic acid in schizophrenic sweat. This finding has not yet been confirmed independently by other workers nor has its presence in other psychiatric patients been investigated. The adrenochrome hypothesis has evolved into the aminochrome-melanin hypothesis. Dr M. Altschule (Harvard Medical School) said that these unstable compounds are rapidly converted to melanin by everybody and, regardless of whether schizophrenics make more aminochromes or not, the fact is that at least one of their tissues, blood, is abnormally sensitive to the toxic effects of these compounds.

The term "orthomolecular psychiatry" introduced by Professor L. Pauling in 1968 has taught American psychiatrists to appreciate a principle well known to scientists. Dr R. Maclean (Vancouver) defined orthomolecular psychiatry as the treatment of mental disease by the provision of the optimum molecular environment for the mind especially the optimum concentrations of substances usually present in the body. Reports which followed testified that large doses of vitamins together with other therapy can improve the functioning of the brain in a variety of conditions. Successes with both children and adult psychiatric patients were reported by Dr A. Cott (New York) and Dr D. Hawkins (Nassau Medical Health Research Center, Long Island). Dr A. Hoffer (Saskatoon, Saskatchewan) outlined the treatment which is based on administering vitamin B3 in combination with other vitamins. The over-regulating mechanism of the body in various forms of stress results in hypoglycaemia and although this is not specific for schizophrenia it is common in schizophrenics and ought to be treated, said Dr R. Meiers (Belmont, California).

REACTOR TECHNOLOGY

Structural Mechanics

from a Correspondent

REACTOR technology requires a synthesis of many skills. The ability to deal with the structural mechanical problems which arise is vital to achieving economic, reliable and safe exploitation of nuclear power. For the first time, largely due to the inspiration of Professor Dr Ing T. A. Jaeger (Bundesan-

stalt für Materialprüfung (BAM)), more than nine hundred delegates from the many disciplines involved in reactor technology met in West Berlin from September 20 to 24 to discuss these problems.

Introductory lectures allowed comparisons to be made between the different strategies applied to power reactor development within the European Community, in the United States and in the United Kingdom. The development of the different types of reactor systems was traced with particular emphasis on the ensuing mechanical-structural requirements.

The rapid progress of reactor technology has given rise to many structural problems which are consequent upon the use of sophisticated materials for various reactor system components. These components are required to operate under severe loading and hostile environmental conditions, and the analyses that must be made range from fuel element and cladding performance under mechanical thermal and irradiation, containment requirements, involving prestressed concrete steel lined vessels or heavy section steel vessels, to dynamic response characteristics of complete power installations. This stimulus has resulted in tremendous strides being made in the area of computational methods. It was underlined by the work of Professor J. H. Argyris (Stuttgart University) and others who have found that with the present generation of large computers it is quite possible to analyse almost any component, however complex, under virtually any loading conditions.

The basis of this development is the "finite element" method which, as the name suggests, splits up the problem into a large number of simpler standard elements which are combined in a suitable way. It has frequently proved to be the only feasible method in complex situations. There are, nevertheless, obvious limitations on cost and there remains the implementation of these procedures (or others) to produce the information required for design. Unfortunately analyses seem to have progressed faster than information on material response and it is not yet agreed how the working life of a structure can be predicted on the basis of failure criteria particularly in complex situations where irradiation damage, fracture and creep may interact. Similarly, analyses can only be as good as the input data they receive for situations such as accident conditions, earthquake disturbances or blast loading. It was emphasized by Professor R. K. Penny (University of Liverpool) that material and component testing of the correct type is essential and should be an interactive process with analyses in the overall optimization of design problems.

Politics of Nuclear Power in the United States

RICHARD WILSON

Department of Physics, Harvard University

In this article the issues surrounding nuclear power generation in the United States are critically examined and some of the misunderstandings are clarified.

NUCLEAR power came of age in the United States in about 1967. At that time about fifty large, new nuclear power stations were started. This dramatic switch from fossil fuel power came because the development of the power reactors had reached a point at which the cost became competitive with fossil fuel power. Two commercial experiments had been constructed and had worked well since 1961—a pressurized water reactor (PWR) at Rowe, Massachusetts, and a boiling water reactor (BWR) at Dresden, Illinois. In spite of rising construction costs, nuclear power remains competitive; oil and gas are beginning to run out in the USA and their prices are rising rapidly.

The American public was, however, slow to react. Scientists who had been arguing in favour of nuclear power—because it is cleaner than fossil fuel power—were pleased but took no particular notice or action. But during 1970 the public became alarmed as the full extent of the US nuclear power programme was made apparent. Until a few years ago, few public hearings in the USA on any subject were well attended and construction permits were obtained for these power stations without too much difficulty. But in 1970 the public began to question the wisdom of these plants both in hearings dealing with construction permits and in those dealing with operating licences.

The reactors being installed in the USA at present are of two types, both using enriched uranium fuel (up to 7% ^{235}U) with water as a moderator. In the PWR, water heated by the reactor produces steam in a heat exchanger inside the containment vessel and there is little release of radioactive gases. But, to make them easy to control, boron is added to the primary water in quantities which diminish as the fuel is consumed; this produces tritium which is released and added to the water effluent. The heat exchanger is omitted for reasons of economy in the BWR and the steam condenser after the steam turbine must be evacuated to achieve high efficiency. Air enters the condenser through leaks in seals and must be released, carrying with it a little radioactivity. The thermal efficiency of these stations is about 30%, compared with 40% for the advanced gas cooled reactors.

The first attacks on the nuclear power industry can be considered as a continuation of the attacks on the Atomic Energy Commission (AEC) because of weapons testing; the hazard to the general population due to radioactivity from weapons had been dramatized by Linus Pauling, Ernest Sternglass and others. The dose commitment from past tests (to the year 2000) of the US population is about 60 mrem (about 2 mrem a year). This is small, and it could be argued that it is tolerable but most of the scientists who were willing to argue in this way wanted to stop weapons tests anyway. Now the situation is different—Dr Sternglass has been joined by Dr John W. Gofman of the University of California and applies the same arguments to nuclear power. Dr Gofman argues^{1,2} that if the whole population were exposed to the

maximum allowed radiation as given by the International Commission on Radiological Protection (ICRP) guidelines (whole body dose 170 mrem per year), then between 9,000 and 110,000 more cancer deaths would be caused each year (depending on his assumptions). It seems probable from the recent work of Dr Alice Stewart at Oxford³ that the unborn foetus is much more sensitive to cancer than is the adult; the probability of cancer induction also seems to remain linear with dose even at low doses. And cancer cases keep appearing for years after the insulting radiation dose. These findings seem so reasonable that, once understood, they are quickly accepted, although some would quote numbers a factor of five smaller than those of Gofman⁴. Dr Gofman's attacks on the AEC were made in a polemical style which delayed recognition of the issue, and brought forth an inept response from the AEC. Dr Storer⁵, a pathologist, argued that there is non-linear behaviour at low doses—a proposition unsupported by reliable experiment and contrary to sound public policy. Moreover, the AEC unnecessarily defended the ICRP guidelines, and failed to define their admonition "as low as practicable".

Restricted Releases

The real answer is only just becoming apparent. Nuclear power now produces much less than 170 mrem average exposure a year. After intervention by scientists in Minnesota (led by Dr Dean Abrahamson) the Monticello reactor is being operated with releases restricted to less than 10% of the AEC regulations (10 CFR/20) and a simple computation shows that the average dose to the public within twenty miles will be less than 10^{-4} of 170 mrem a year. The State of Minnesota supported this restriction and tried to enforce it legally; Congress had, however, specifically given the AEC the right to set radiation regulations for nuclear plants, and this has been upheld by the US Federal Court. But in practice Minnesota will have its way. In Vermont, the Vermont Yankee Power Company signed an agreement with several conservation groups to abide by the lower standards of the Vermont Bureau of Public Health. Even so, Vermont Yankee is now entering what promises to be a long operating licence hearing; operation of the power station was scheduled for December 1971 and will certainly be delayed at a cost of \$80,000 a day.

At Plymouth, Massachusetts, the Advisory Committee of the AEC on Reactor Safeguards has insisted on the earliest possible installation of a holdup filter to stop gases with short half lives. All existing BWRs will now be modified in a similar way. In May the AEC introduced new design criteria for light water reactors; the design must limit the worst fence post dose to 10 mrem per year, and the designed equipment must be used. Only two old reactors fail to meet this criterion now. The newer ones give lower radiation by a factor of as much as 10. The reprocessing plants are planning to hold up even ^{85}Kr within a few years.

The AEC is not the only agency to hide behind the ICRP and is not the worst offender. In spite of excellent practices by some of the leading hospitals, the average radiation dose to Americans due to diagnostic X-rays was 95 mrem in 1970 (ref. 6), twice the 1964 dose and seven times the average British dose. The American Medical Association has been silent.

Thermal Problems

Environmental groups have forced through important changes in thermal pollution standards. This is an old story in England where the River Trent, for example, which is small and sluggish by US standards, has 12,000 MW of installed generating capacity, more than any comparable river in the USA; cooling towers abound. Not only do nuclear power stations give out twice as much heat as fossil fuel power stations, but nuclear power is at present less efficient and there is no loss of heat up the chimney.

The first nuclear power station where this problem was faced was Connecticut Yankee at Haddam's Neck near the mouth of the Connecticut River. Cooling is done with river water, but a study of the effects of the heating is under way⁷. Other power companies have not been so fortunate; further up the Connecticut River at the Vermont Yankee station mechanical draught cooling towers which can work on a closed cycle have had to be installed. In the region of the Great Lakes, the Lake Michigan Environmental Conference wishes to set and enforce a rule allowing a maximum water temperature rise of 1° F. This matter involves many states and is being considered by the director of the newly formed US Environmental Protection Agency (Mr W. Ruckelshaus). Some scientists think the rule is extreme and unnecessary⁸. Lake Michigan, for example, has an area of 10,000 miles², and the heating from each power station is equivalent to solar heat over 1 mile². If the water is spread over the whole surface of the lake, all power stations up to the year 2000 could be accommodated⁹ with a temperature rise of only 0.1° F, so the problem becomes one of achieving this uniformity. Particularly at issue is the fate of eight power stations under construction—the power companies estimate a cost of \$200 million to add cooling towers at this stage.

The AEC was sensitive to criticisms of excessive political power and had no expertise in this field; hence it tried to avoid considering thermal pollution. It tried merely to "certify" the state or other federal standards and to consider only radiation and reactor safety in its hearings. Many people have tried to persuade the AEC to "hand over" all the hearing and compliance procedures to the Environmental Protection Agency. The Environmental Protection Act, as interpreted in July by the US Court of Appeals in the consideration of a nuclear power plant at Calvert Cliffs in Chesapeake Bay, insists, however, that the AEC has the right and duty to control all environmental matters. This will involve more delays—especially for those power stations which are now involved in operating licence hearings. It is ironic that conservation groups have forced the AEC into taking on more political power at a time when others accuse it of having too much.

Accidents and Safety

The third issue, least emphasized but most important politically, is that of safety. Each reactor makes enough plutonium in a year for thirty atom bombs, and radioactive material in proportion. Could an accident occur which would spread this over the countryside? Clearly a direct hit from a hydrogen bomb would do so, but what else?

If there is a catastrophic pipe failure in a light water reactor (which in itself is unlikely ever to happen) the depressurization (blowdown) will lead to loss of coolant (and moderator). Emergency core cooling must immediately be applied to prevent fuel melting and release of radioactivity. In a typical PWR 17,000 gallons of borated water held under pressure would immediately be injected into the vessel. But small scale (9 inch instead of 18 foot) tests held at the National Reactor Testing Station at Idaho in November of 1970 showed that the water injected during the blowdown fails to stay. Although calculations suggest that the system behaves better in the full scale reactor, the AEC first stalled on all new operating licences, and then in June issued tighter standards. The utility company must prove that the reactor can cope with

a double-ended guillotine break, even if all water injected during the blowdown is lost. Superficially this does not affect the BWRs or the two helium gas-cooled reactors, but a few scientists argue¹⁰ that none of the old safety arguments can be trusted, and that no new reactors should be licensed until a new independent study has been made.

This aspect of nuclear power is probably the least well comprehended. To understand this, we must understand other serious accidents. One can talk about the 88,000 direct coal mining deaths since 1907 (in addition to black lung and various other diseases) or the 2,000 people killed when a hydro-electric dam gave way near Belluno, Italy, in 1963, but there is no public understanding of such comparisons and even less acceptance. The US public will probably not accept nuclear power finally until there is a complete understanding of all types of accident—and an understanding which can be simply expressed.

The power companies are now becoming reconciled to long hearings on any power station. The Long Island Lighting Company has been trying to obtain a construction permit for a BWR at Shoreham, but this has already taken 12 months of hearings in spite of the help of some local citizens who work at Brookhaven National Laboratory. The President's Office of Science and Technology¹¹ has recommended that site searches take place in public over several years. This is a good development, but the USA will be faced with power shortages in the next few years while the power companies adjust to the new situation. Westinghouse is now designing reactors on barges to be floated 20 miles offshore.

The AEC, for its part, must quickly undertake the vital task of restoring public credibility. As a result of many factors, and in particular the SST and the Vietnamese war, the American public have learned to distrust the experts in Washington. The AEC has been talking for twenty years, but few members of the public have been listening. Now people are listening but the AEC must learn how to talk clearly and simply.

One possible consequence of failure to reorganize promptly is apparent in New York. Faced with forthcoming power shortages, and a completed nuclear power station which is not allowed to operate, Mayor Lindsay has overruled many of his advisers, and has allowed the installation of 2,000 MW of gas turbine power on the East River in New York, which now operates every hot summer afternoon. These are little better than jet engines blowing against windmills. The disadvantages in noise, thermal pollution, and consumption of expensive scarce fuels are obvious.

In Midland, Michigan, where a nuclear power station is held up by intervention in public hearings, Dow Chemical Company announced the transfer of part of its operation to New Orleans where power is cheaper. Local citizens took out a full-page advertisement attacking the interveners. Conservation societies now have great political power in the US—particularly on nuclear questions; they must use this power wisely.

¹ Gofman, J. W., *Testimony in Hearings Before the Joint Committee on Atomic Energy, Ninety-first Congress of the United States, Second Session on Environmental Effects of Producing Electric Power*, Part 2 (1), 1388 (1970).

² Gofman, J. W., *Proc. April Meeting of the American Physical Society, Washington DC* (1971).

³ Stewart, A. M., and Kneale, G. W., *Lancet*, i, 1185 (1970).

⁴ Hamilton, L. D., *Proc. April Meeting of the American Physical Society, Washington DC* (1971).

⁵ Storer, J. W., *Testimony in Hearings Before the Joint Committee on Atomic Energy, Ninety-first Congress of the United States, Second Session on Environmental Effects of Producing Electric Power*, Part 2 (1), 2186 (1970).

⁶ *US Public Health Service Report* (in the press).

⁷ Merriman, D., *Sci. Amer.*, 222 (5), 42 (1970).

⁸ Gustafson, P. F., *Bulletin of the Atomic Scientists* (March 1970).

⁹ Asbury, J. G., *Effects of Thermal Discharges on the Mass/Energy Balance of Lake Michigan* (Argonne National Laboratory Report ANL/ESI).

¹⁰ Union of Concerned Scientists, Cambridge, Mass., press release (July 1971).

¹¹ *Electric Power and the Environment* (Office of Science and Technology, 1970).

The Oxford Botanic Gardens: 1621 to 1971

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On July 24 this year, the 350th anniversary of the old Physic Garden in Oxford was celebrated by the university. A guide to the old and new gardens has been published by the Oxford University Press and an exhibition set up in the Divinity School to mark the event.

LORD DANBY, the founder of the Oxford Botanic Garden, was one of the great adventurers of the Elizabethan age. Sir Philip Sydney took him as a page on his fatal expedition to the Netherlands in 1586 and soon afterwards he was exiled by Elizabeth to France for a murder; his brother was later beheaded for rebellion. This was the violent phase when he served as the model for the Romeo of Shakespeare¹. But after these youthful indiscretions, Henry IV of France befriended him, James I made use of him, and in good time he found himself an Earl and a Knight of the Garter. It is in this respectable phase that his bust appears adorning the Inigo Jones gateway of his garden (Fig. 1).

Danby was prompted to set up his garden in Oxford by Thomas Clayton, the professor of medicine. But no doubt it was his own experiences which made him demand a wall to defend the garden against friends and foes as well as against frost. The wall took twelve years to build and proved strong enough to meet all these dangers. Civil war in the seventeenth century, a bridge and road scheme in the eighteenth century, and an expansion plan in the nineteenth century were all withstood by Danby's wall.

Jacob Bobart had been given a 99-year contract as keeper of the garden for himself and his heirs in 1637, a contract which allowed a far-sighted policy. By 1641 he had produced the necessary heir and by 1648 he had issued a catalogue of the 1,600 plants that he had collected. Many had been grown from seed sent by other gardens and, in about 1666, he or his son raised the hybrid plane and recognized it as a hybrid. It is now known as the London plane but its finest specimens are still to be seen in Oxford.

Evidently the garden flourished for the 82 years that it was managed by the Bobarts, in spite of financial difficulties. Loggan in 1675 shows their orangery and the four gated enclosures which they had made for their beds. Here Pepys and Evelyn came with other inquisitive visitors; here the physicians learnt their drug plants, among them Radcliffe who built the Camera with the profits of his profession. Here, too, Robert Hooke must have sought out the plants for his *Micrographia*. And here the younger Bobart began his collaboration with Robert Morison².

Morison came from Aberdeen, fought for Charles II and followed him into exile, and then studied several sciences in Paris. He was uncouth in appearance but the King liked him and, after his restoration, made him his own physician as well as his own gardener. Sponsored by Charles in 1669 he undertook the additional duties of professor.

Morison brought with him from France several "striped" herbs and trees including the mottled sycamore. They still survive in the garden, embodied in the great classified collection of variegated plants. Next, in collaboration with the younger Bobart, he began a detailed account of the flora of Oxfordshire; the joint experience of the two men of raising plants from seed gave them a new point of view. Their recognition of the pro-



Fig. 1 The Inigo Jones gateway

cesses of sexual reproduction (which they shared with John Ray) led them to use the reproductive rather than the vegetative organs as the basis of classification and helped to make explicit the daring idea of genetic relationship, the slow advance of which was to cover the next three hundred years.

When Morison died prematurely in London in 1683, knocked down by a coach in the Strand, his salary, which was to have been paid by the King, had long been in arrears. The younger Bobart then became the unpaid professor and had to complete their joint work alone. It was this devoted work which must have attracted the attention of William Sherard, then an undergraduate in law at Oxford. Later Sherard in his capacity as British consul at Smyrna from 1703 to 1717 acquired a fortune and was able at last to indulge his botanical interest. He then began to make extensive journeys collecting plants, a library and, most important of all, a collaborator—Johann Dillen or Dillenius.

Dillenius was one of a succession of German botanists who excelled as draughtsmen and, by their comprehensive labours, made it possible to establish the systematic ideas of Ray, Morison and later Linnaeus. Sherard first installed Dillenius as Foreign Secretary of the Royal Society and then set to work to establish him as professor at Oxford, with an endowment both for the chair and for the garden. He was careful to exclude the danger, endemic in Oxford at that time, of any connexion with the Church.

The immediate fruit of Sherard's gift was the production of Dillenius's *Historia Muscorum*, the most thoroughly illustrated early account of the lower plants; in this way the practice of plant illustration took root in Oxford. The next professor, Humphry Sibthorp, did little himself. He may, have had Joseph Banks in the audience of his single lecture but we have to suppose that it was the garden and not the lecture that later inspired Banks to establish the Royal Gardens at Kew. Sibthorp's artistic interests are shown, however, by his acquisition not only of seed of the Chinese ginkgo but also of a Chinese painting of the tree, now on show at the Divinity School in Oxford. Accordingly it was a botanical artist, Georg Dionysius Ehret, that Sibthorp engaged as head gardener. Ehret stayed only a year for he would not take orders from Sibthorp. He made his way to London, but his descendants left their mark in Oxford. The fourth generation gave us Sir Arthur Evans, Keeper of the Ashmolean Museum, by whose enterprise the plant decorations at Knossos were to be discovered.

The urge to collaborate with artists and to seek them out abroad descended to Sibthorp's young son for whom he made way in 1784. For John Sibthorp arrived in Vienna to find that the fifth century illustrations of Dioscorides had recently been brought from Constantinople and, by order of Maria Theresa, had been engraved and some twenty-five copies printed. Equipped with one of these and with ample funds, he was accompanied by yet another German artist, Ferdinand Bauer, as he made his way to Greece with the object of having every plant in the classical catalogue drawn again from nature and printed and published in the modern style. He and Bauer later extended this original purpose, first to include the general flora of Greece and then the fauna as well.

Before this plan could be fulfilled Sibthorp died after only two journeys in Greece. But he had endowed the publication of his work. Bauer completed the illustrations, and after forty-four years all the 960 coloured plates had appeared in the ten volumes of his splendid *Flora Graeca*.

It was now 1840, however, and times had changed. The study of physic and practice of draughtsmanship had ceased to be the mainsprings of plant science. New possibilities had arisen and a new kind of teacher appeared in Oxford to seize the opportunity. This was Charles Daubeny, an amiable man who was willing to expound any science known at the time—except theology. He was elected in succession to the chairs of chemistry, botany and rural economy and he held them concurrently. Quick to learn, it was said he had picked up what

botany he needed from the De Candolles during a few months in Geneva. He was neither profound nor original and he did not know enough to be pedantic. But he was willing to try other people's ideas and eager to teach them. The result was that he and his contemporary Acland proved to be the most effective of university innovators.

The young Daubeny realized the value of Liebig's experiments with mineral fertilizers as soon as he heard of them. He at once tried them out on plots in what he called the Botanic Garden. His pupil John Lawes took the idea home with him and founded Rothamsted. Later, an older Daubeny at once saw the merits of evolutionary theory and when the British Association came to Oxford in June 1860 it was Daubeny who opened the Darwinian debate. The title of his paper (*On sexuality in plants*) pointed back to Morison and forward to the present day³. And it was Daubeny who concluded the debate with a party that evening at his house in the Botanic Garden: in the heart, as we may say, of enemy country.

Daubeny and Acland saw the need for unifying the sciences and fitting them into a larger scheme of education. It was a development of this vision when, within a few years of his death in 1867, Lawson and Lankester were joining together to teach a course in biology in the Botanic Garden. But this development was not to prosper. The craving for specialization in teachers, the demand for independence of professors, but above all the great fear in the university of expanding science, turned the current of opinion in the opposite direction. In these circumstances the university appealed to Dr Hooker², the director of Kew, for guidance. It was relieved to learn from him that botany in Oxford could well stand by itself and could stand for ever on the four acres of the Botanic Garden.

With this guidance, teaching as well as research fell into decay. The attachment of botany to medicine, which could have carried both subjects into a creative experimental period (putting Mendel and Garrod together), remained static for a further eighty years in the forms of an ancient syllabus. In 1883 Lawson emigrated and the Sherardian Chair fell vacant; once again there was no paid teacher or manager in the garden. In 1901 Daubeny's now useless experimental field was sold. And appropriately, forty years later, the superintendent's seed list was still titled in Latin and his office was still lit by the light of a single candle.

Today these setbacks and holdbacks can be seen from a happier position. In 1944 the garden itself was doubled in size and on the new ground there has been arranged a collection of roses whose history of hybridization and selection is now defined by pedigree and also by chromosomes. In 1954 a Genetic Garden, now in the University Parks, was added to display, with materials both for teaching and research, not only the results but the processes of evolution. And in 1969 an anonymous benefactor put a third garden at the disposal of the university, the established and now rehabilitated arboretum of Nuneham Courtenay.

We can now look back at the contrasted succession of naturalists and experimenters who have worked and taught in the Botanic Garden. Through them runs the continuity of the garden itself with its collections of plants, its series of glass-houses (now rebuilt for the seventh time) and its long line of gardeners. Notable among these are the Bobarts and the Baxters—fathers and sons—whose skill has been passed on from generation to generation and who have maintained the garden, disseminating their knowledge and materials worldwide through catalogues, exchange lists and guides. In the future, even more than in the past, we may expect that the practical wisdom of the garden and of the gardeners will continue to hold together the branching strands not of one but of several sciences.

¹ Rowse, A. L., *The Times*, July 24 (1971).

² *Makers of British Botany* (edit. by Oliver, F. W.) (Cambridge University Press, 1913).

³ Daubeny, C. G. B., *Dictionary of National Biography* (1900).

General Model for the Structure of all Myosin-containing Filaments

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It is suggested that the way in which the molecules are packed is common to all types of myosin filament. This leads to quantitative predictions about the component molecules.

It has been known for some time that thin actin-containing filaments, all of basically the same structure, occur in all the types of muscle so far studied¹⁻⁶. These muscle types include vertebrate striated muscle (VStM), vertebrate smooth muscle (VSmm), insect fibrillar flight muscle (IFM) and molluscan muscles (MM). On the other hand, it is generally accepted that the myosin-containing filaments in the muscles mentioned are structurally quite different⁵⁻¹¹ apart from the following points of similarity. (i) All the myosin filaments are known to have an axial repeat of about 144 Å. (ii) It is thought that the heavy meromyosin portions¹² of the myosin molecules which form the filaments project from the surfaces of the filaments to form cross-bridges which can interact with neighbouring actin filaments^{8,13}. Evidence of such projections has been obtained directly by electron microscopy (refs. 10, 11 and 15-17 and J. V. Small, and J. M. Squire, in preparation) and has been deduced from X-ray diffraction studies of intact muscle^{13,14,18,19}.

The apparent differences between these myosin-containing filaments are considerable. (i) The filaments can either be cylindrical (VStM, IFM and MM) or ribbon-like in shape (VSmm)⁹. The cylindrical filaments vary greatly in diameter and length although they probably all taper at each end, there is probably end to end polarity of the cross-bridges and probably a bare zone half way along the filaments²⁰. The VSmm myosin ribbons have opposite polarity on opposite faces (ref. 17 and J. V. Small, and J. M. Squire, in preparation). (ii) Those myosin-containing filaments which have been analysed structurally (VStM, IFM and VSmm) have projections arranged on nets which apparently have quite distinct symmetry and spacings (except for the common 144 Å axial period) in the different types of filaments. In VStM the myosin projections are thought to be arranged on a two-strand 6/1 $\uparrow$ helix of repeat 429 Å (ref. 8), in IFM¹¹ they are thought to be arranged on a

two-strand 16/3 helix of true repeat about 1150 Å and in VSmm the projections are thought to be arranged on each face of the ribbon in a lattice of approximate dimensions $a=100$ Å (across the ribbon face), $b=150$ Å and $\gamma=74^\circ$ which corresponds to an axial repeat of about 720 Å (ref. 17).

The relationship between projections in these models of the different filaments is shown in Fig. 1. In VSmm it is very probable (J. V. Small, and J. M. Squire, in preparation) that each lattice point in Fig. 1a is associated with the origin of the heavy meromyosin portion of one myosin molecule. In biochemical estimates of myosin content for VStM²⁰ it seems that there may be two myosin molecules associated with each lattice point (see ref. 8, page 418). In insect flight muscle the estimates of myosin content suggest that there are either two molecules per lattice point which seems to agree with Reedy's observations of transverse sections of insect muscle in the electron microscope¹¹ or there are three molecules per lattice point²¹.

It is known that the myosin molecules extracted from different muscles, although different in some respects, have basically the same form¹² and approximately the same molecular weight^{5,22}. They consist of a two-chain α -helical rope in the rod portion of the molecule which is linked at one end to a globular unit (the myosin head)^{20,23,24} comprising two large globular subunits¹² which may be associated with several light chains^{25,26}. One might ask why these similar molecules pack together in such different ways in different muscles. On the other hand, one might consider whether there are alternatives to the existing models^{8,11,27,28,30} for the structures of these filaments which will satisfactorily account for the published experimental data and in which the packing of myosin molecules is basically the same in all types of myosin-containing filament. This communication represents the first step in the exploration of such an idea.

Clues to Basic Myosin Filament Structure

I have quoted results from the analysis of the structure of the myosin-containing ribbons in VSmm^{9,17}. The ribbons and their cross-bridges have been visualized directly by electron microscopy. Optical diffraction analysis of micrographs of thin longitudinal sections in which the planes of the ribbon and the section were parallel has given direct information about the cross-bridge lattice on the faces of the ribbons (ref. 17 and J. V. Small, and J. M. Squire, in preparation). Fig. 1a shows the form of this lattice. We can therefore start with this evidence and use it to help to deduce alternative structures for the other myosin-containing filaments.

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$\uparrow$ The notation m/n helix has been used throughout to denote a helix which repeats after n turns in which there are m residues.

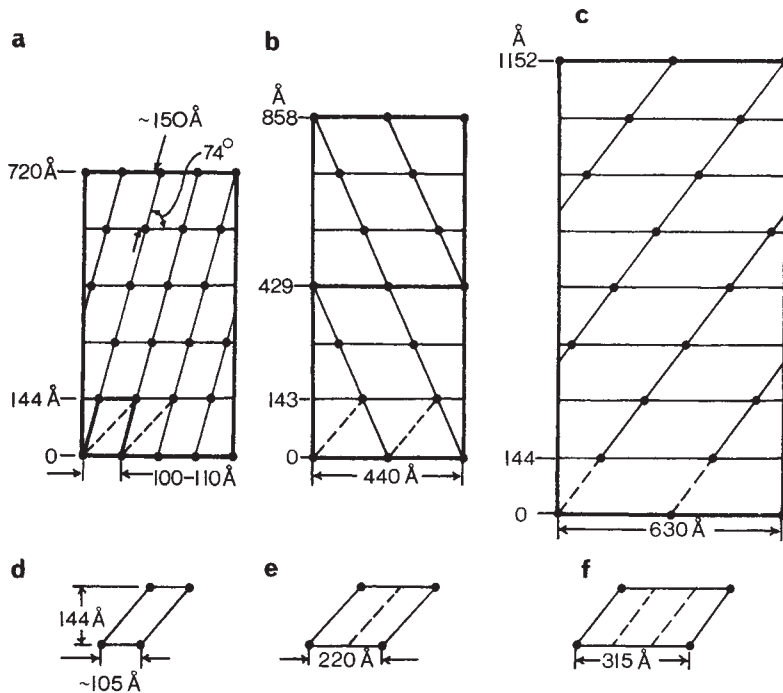


Fig. 1 *a*, The probable lattice of cross-bridges on each face of the myosin ribbons of vertebrate smooth muscle (ref. 17 and J. V. Small, and J. M. Squire, in preparation). *b*, Radial projection of the cross-bridge lattice in the model of the myosin filaments in vertebrate striated muscle proposed by Huxley and Brown⁸. *c*, Radial projection of the model for the cross-bridge lattice on the thick filaments of insect flight muscle proposed by Reedy¹⁰. *d*, *e* and *f* show repeating units in *a*, *b* and *c* respectively and demonstrate how these units are related.

First of all, if we take two of the repeating units (Fig. 1*d*) on the ribbon lattice shown as a dashed line in Fig. 1*a* we generate a repeating unit similar to that in the two-strand 6/1 helix of VStM (Fig. 1*b* and *e*). It is thought that there are probably two molecules per lattice point in the VStM filaments on the basis of two-strand 6/1 helical symmetry⁸. This suggests that a four-strand 6/1 helical structure (Fig. 2) with one molecule per lattice point (as for the ribbons) might be a possible alternative arrangement to the two-strand 6/1 helix. Such a four-strand 6/1 helix would generate the observed repeat of 429 Å, it would have a residue repeat of 143 Å giving the observed meridional X-ray reflexion and in projection at right angles to the filament axis (that is, the view obtained in longitudinal section of muscle²⁹) it would appear almost identical to the same projection of a two-strand 6/1 helix. The only difference in the X-ray diffraction pattern would be that the intensity distribution on layer lines other than the third, sixth, and so on would relate to J_4 and higher order Bessel functions but not to J_2 Bessel functions as for the two-strand 6/1 helix. Using the intensity profile on the first layer line given by Huxley and Brown⁸ it is

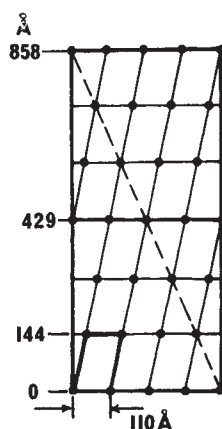


Fig. 2 Possible alternative model for the cross-bridge lattice on the thick filaments of vertebrate striated muscle in radial projection. The bold line outlines a repeating unit similar to that of the lattice in Fig. 1*a*. The dashed line represents one of the four 6/1 helical strands.

possible to estimate (without attempting to fit this curve exactly) that a J_4 Bessel function in this region would relate to a helix of radius about 160–170 Å. These layer lines arise solely from the arrangement of cross-bridges on the myosin filament⁸ and most of the mass of the cross-bridges is located in the heavy meromyosin regions which form the bridges. It is therefore not unreasonable that most of the diffracted intensity on first and second layer lines should relate to a radius of 160–170 Å which lies between the surface of the filament backbone (radius about 70 Å) and the surface of the actin filaments (approximate radius 200 Å)⁸. In this case it would seem that the myosin heads are quite close to the neighbouring actin filaments. It will be shown elsewhere that the proposed model can account for the observed exchange of mass from myosin to actin when a relaxed muscle goes into rigor¹³. It is therefore possible that the four-strand 6/1 helix is a satisfactory alternative model for the myosin filament in VStM.

Next we can consider the structure of the myosin-containing filaments in insect flight muscle. This time Fig. 1 shows that three repeating units of the ribbon lattice (*d*) would give a repeating unit rather like that on the proposed two-strand 16/3 helical model for these filaments (*f*). If we take the estimate of myosin content²¹ which gives three molecules per lattice point on this model (and not two), then an alternative model with the same helical symmetry but with six molecules equally spaced around the myosin filament on one level seems possible. This would generate a six-strand 16/3 helix (Fig. 3) with a long repeat of about 1150 Å. The experimental evidence from which the original model was deduced was from X-ray diffraction work¹⁶ and electron microscopy^{10,11,16}. The electron micrographs of longitudinal sections of insect flight muscle were analysed by optical diffraction and reflexions which were orders of a repeat of 1150 Å were observed. From the X-ray diffraction results it was deduced that the residue repeat of the cross-bridge lattice on the myosin filaments is about 144 Å. These results taken together led Reedy to propose the two-strand 16/3 helical model for the myosin filaments. The alternative six-strand 16/3 helix also has a repeat of 1150 Å and a residue of 144 Å and would equally well account for these results.

The other experimental evidence which needs to be explained is the appearance of “flared-X” configurations of the projections in Reedy’s very elegant electron microscope work on serial

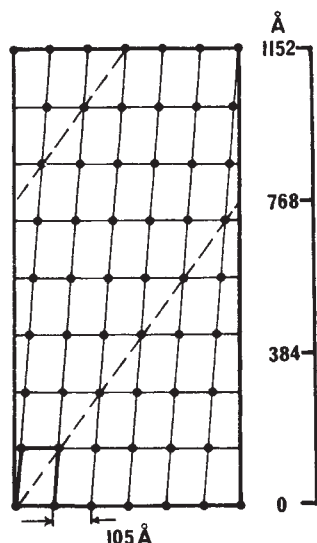


Fig. 3 A possible model for the thick filaments in insect flight muscle; radial projection of units arranged on a six-strand $16/3$ helix of repeat 1152 Å. The dashed lines follow one and a half turns of the $16/3$ helix.

thin transverse sections of glycerinated insect flight muscle¹¹. These seem to show that there are at most only four cross-bridges with origins within one repeat of 146 Å along the myosin filaments. But Reedy states¹⁰ that the "flared-X" configurations cannot be convincingly demonstrated in sections of relaxed insect flight muscle where presumably the myosin heads are not necessarily cross-linked to actin filaments. It is therefore possible that cross-bridges are only visible in Reedy's micrographs if they were cross-linked to actin before fixation. In this case the proposed six-stranded model would require that even in a rigor muscle (in which it is commonly assumed that all the cross-bridges are attached to actin) only four out of six of the cross-bridges on one level are cross-linked. These would then form the flared-X configurations seen in Reedy's micrograph and the remaining cross-bridges would not be preserved and visualized. Evidence for this will be given elsewhere (J. M. Squire, in preparation). It is therefore possible that a model for the myosin filaments in insect muscle in which there are six cross-bridge origins on one level may adequately account for the existing experimental data on the structure of these filaments.

Implications of Alternative Models

I have now arrived at the situation where the structure of the myosin-containing filaments in VSmM, VStM and IFM might be explained in terms of models with basically the same distribution of myosin heads on the surfaces of the filaments. If it is assumed that the myosin molecules pack together with about 1000 Å of the rod portion of the molecules associated with the backbone of the filaments (the remainder forming the cross-bridges) and also that the myosin rods are close packed in the backbone with a separation of about 20 Å, then the suggested arrangements of projections would require the rod portions to pack into a surface layer of myosin molecules of thickness about 30–40 Å. This may of course vary for cylindrical filaments of different total diameter and will be slightly larger for the smallest filaments. Taking reasonable values (which can only be approximate) for the dimensions of the backbones of the filaments being considered (about 140 Å diameter in VStM^{8,30}, about 200 Å diameter in IFM^{10,11} and the thickness of myosin ribbons in VSmM about 145 Å (ref. 17

and J. V. Small, and J. M. Squire, in preparation)) then the filament profiles drawn in Fig. 4 are obtained. An approximation has been made here in that differences in filament structure in the bare zones and tapered ends of the cylindrical filaments have been neglected. The diameters given do not relate to the bare zone but to the regions on the filaments from which most of the cross-bridges originate. In these regions the filaments would all consist of a core of protein other than myosin surrounded by a layer of myosin of thickness 30–40 Å. The observations of "hollow" filaments (or at least filaments which have a central core with different staining properties from the periphery) in muscle cross-sections are quite numerous^{16,31} and favour this sort of model. Szent-Györgyi *et al.*³² have also suggested that there may be a constant surface area per cross-

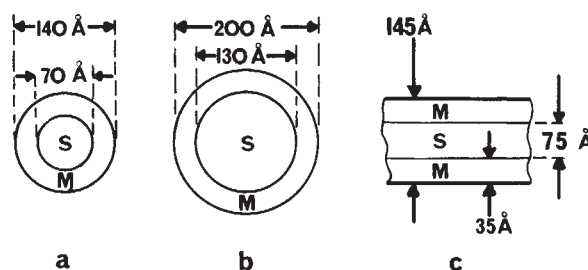


Fig. 4 Profiles of different myosin-containing filaments assuming the myosin rods are confined to a layer of thickness 35 Å on the surface of the filaments. *a*, Vertebrate striated muscle filaments; *b*, insect flight muscle filaments; *c*, ribbons in vertebrate smooth muscle. The myosin projections do not form part of this layer.

bridge (and therefore a constant thickness of myosin) on the surfaces of the paramyosin filaments of different molluscan muscles. This is discussed in the next section.

If we now consider just those filaments which are cylindrical in shape we can calculate approximately the percentage volume of the filament backbone which will be taken up by the core in filaments of different diameter if the myosin is all in a surface layer of thickness, say, 35 Å, and it is assumed that 60% of the mass of myosin molecules is in the projections and does not form part of the filament backbone. Fig. 5 shows this percentage volume (V_c) plotted as a function of the diameter of the backbones of the filaments. The two points which would correspond to the models illustrated in Fig. 4 for VStM and IFM are shown on this graph together with points related to other models for these filaments.

Paramyosin-containing Filaments

If the ideas mentioned above have any basis in reality, then the paramyosin-containing filaments in molluscan muscles should have the same sort of structure with a 35 Å layer of myosin muscles surrounding an inner core of paramyosin. In this case we are in the fortunate position of having experimentally determined values for the proportions of paramyosin (P) and myosin (M) in particular muscles. We can therefore plot several points on Fig. 5 which correspond to experimental results for filaments of widely varying diameter. Recent estimates of the ratio P : M by weight in different muscles are 25% : 75% in *Pecten* striated adductor muscle (private communication of preliminary results by P. M. D. Hardwicke, and J. Hanson); 50% : 50% in *Venus mercenaria* white adductor muscle³², 33% : 67% in the translucent adductor muscle of *Crassostrea virginica*³² and finally in *Crassostrea* opaque adductor muscles 57% : 43%³² or 75% : 25% (private com-

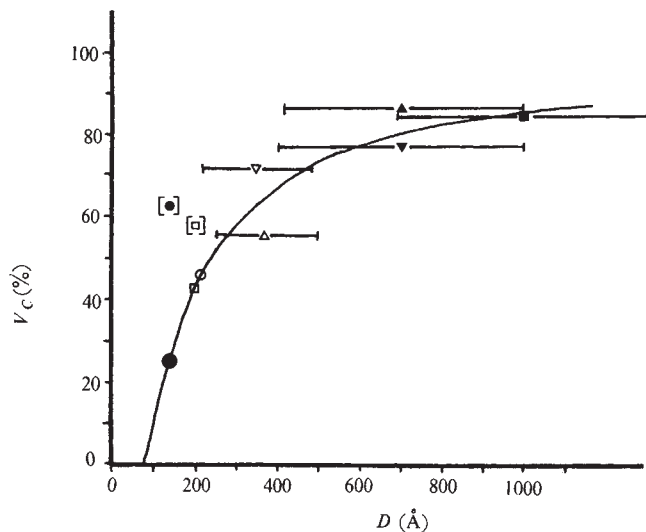


Fig. 5 The bold line represents a plot of the volume of the core (V_c) expressed as a percentage of the total volume of cylindrical myosin-containing filaments of different diameter (D), assuming that there is a layer of myosin of thickness 35 Å on the surfaces of these filaments, and that 60% of the mass of the myosin molecules is in the cross-bridges and does not form part of the backbone. ● and □ represent the appropriate points for filaments in VStM and IFM on the basis of the models shown in Figs. 2 and 3 respectively. The same symbols in brackets refer to existing models^{8,10} for the myosin filaments in the muscles. The remaining points represent experimental results obtained from the relative amounts of paramyosin and myosin in different molluscan muscles. Error bars on these points represent the standard deviation of the filament diameters about the mean assuming the sort of distribution of filament diameters observed in *Crassostrea* opaque adductor muscle³⁴. ○, *Pecten* striated (P. M. D. Hardwicke, and J. Hanson, personal communication); ▽, *Venus* red³²; ■, *Venus* white³²; △, *Crassostrea* translucent³²; ▼, *Crassostrea* opaque³²; ▲, *Crassostrea* opaque adductor muscles (P. M. D. Hardwicke, and J. Hanson, personal communication).

munication from P. M. D. Hardwicke, and J. Hanson). In *Pecten* the paramyosin filaments have a diameter of about 200 Å to 250 Å (except in the bare zone). In the other muscles the paramyosin filaments can have a variety of diameters. Published estimates of the range of diameters (which also include the effects of the tapering of the filaments) are as follows: 200 Å to 500 Å in *Venus* red muscle³³, 500 Å to 1500 Å in *Venus* white muscle¹⁵, 150 Å to 600 Å in *Crassostrea* translucent muscle¹⁵ and 200 Å to 1500 Å with a peak of the distribution at about 700 Å (standard deviation 300 Å) in *Crassostrea* opaque adductor muscles³⁴. Although these sizes cannot be

taken too literally it is probable that "average" values of the diameter will reflect the relative filament sizes in the different muscles. Fig. 5 shows how the experimental figures relate to the model and demonstrates that in each case there is sufficient paramyosin to fill the core of the different filaments.

Although there is obviously considerable uncertainty in the results, we can conclude from the general agreement between the theoretical curve and the plotted points that, at least, the model is consistent with the available data. This serves to emphasize one point made in Szent-Györgyi *et al.*³² that the surface area per myosin head is probably a constant feature of all paramyosin-containing filaments. As in the other filaments this area would be about $144 \text{ Å} \times 100\text{--}110 \text{ Å} = 15,000 \text{ Å}^2$.

On this basis one can suggest possible packing arrangements for the myosin heads on the surfaces of different paramyosin-containing filaments. The paramyosin in these filaments is known to pack to give a surface appearance³⁵ well known as the Bear-Selby net (Fig. 6a). The axial repeat of the net is always about 720 Å but the "a" spacing varies greatly in filaments from different muscle types and also with the diameter of the filaments within one muscle type⁷. Fig. 6 shows the Bear-Selby nets of filaments in four different molluscan muscles (for details see caption) and illustrates how these nets can be subdivided very simply (assuming all the lattice points in the Bear-Selby net are equivalent) to give repeating units of area about 15,000 Å². The mean "a" spacings are obtained mostly from X-ray diffraction results³⁶ and are assumed to be similar to the "a" spacings on the surface of the paramyosin core of the filaments⁷. It is therefore quite possible that the lattices drawn in Fig. 6 represent the packing distribution of myosin on these filaments. It should be noted, however, that in VSmM evidence has been obtained that the filament backbone and the cross-bridge lattice on the myosin ribbons do not have the same axial repeat (J. V. Small, and J. M. Squire, in preparation). In spite of this the present model would require that the myosin molecules pack with a side-spacing of about 100 Å. Evidence in support of this is shown in Fig. 7 where a micrograph of a paramyosin filament ("a" spacing, 630 Å) from the anterior-byssus retractor muscle (ABRM) of *Mytilus* is shown by self-convolution³⁷ to exhibit a lateral repeat of about one fifth of the "a" spacing (that is, 120–130 Å) which is generated by a rectangular net of the sort drawn in Fig. 6e.

Discussion and Predictions

So far I have tried to show that it may be possible to interpret the existing experimental data on the form of the myosin-containing filaments in different muscles in terms of one basic structure. The details of this interpretation will be given later

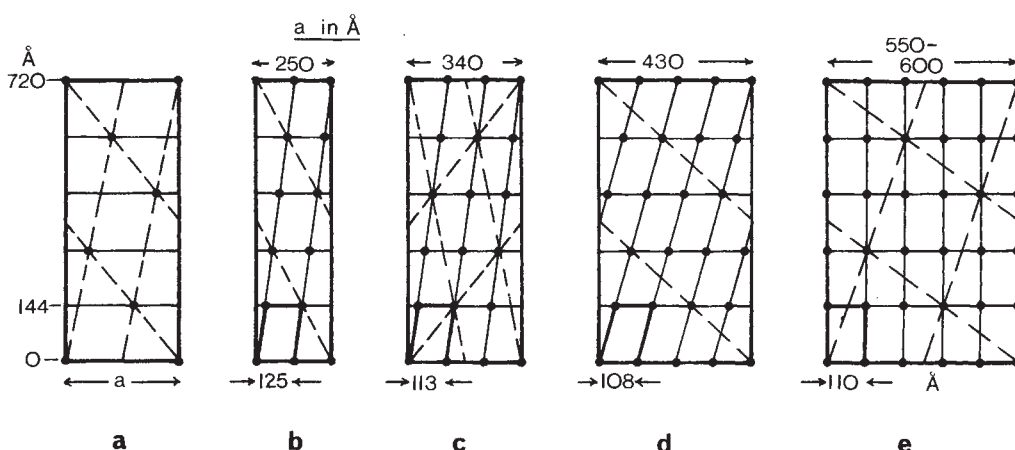


Fig. 6 a, The lattice (Bear-Selby net) on the surface of paramyosin filaments from which the myosin has been removed; b-e represent subdivisions of the Bear-Selby nets on the surfaces of paramyosin filaments in different molluscan muscles to give repeating units of area 15,000 Å². It has been assumed that lattice points in the Bear-Selby nets are all equivalent. b, *Venus* adductor muscle; c, *Crassostrea* adductor muscle; d, *Mytilus* adductor muscle; e, *Mytilus* ABRM.

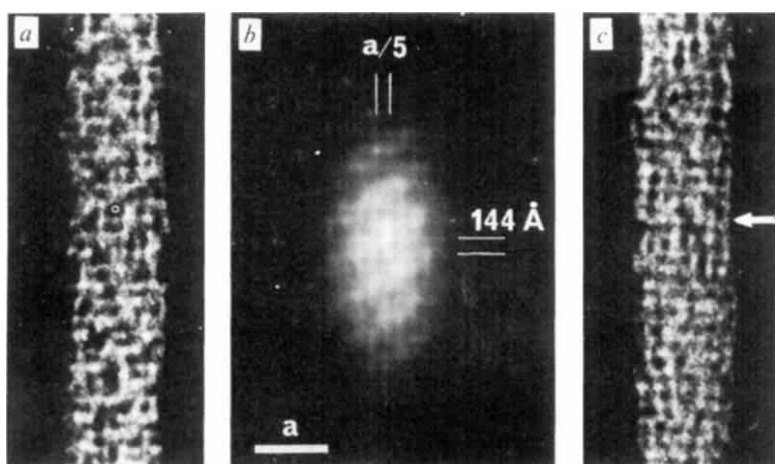


Fig. 7 *a*, Electron micrograph of part of a negatively stained paramyosin filament (courtesy of Professor J. Lowy) from *Mytilus* ABRM; *b*, self-convolution function of part of the filament in *a* which shows that "a" spacing of the Bear-Selby net on this filament is clearly divided into five sub-periods; *c*, another region of the same filament as *a* showing a regular near-rectangular arrangement of subunits (compare Fig. 6e).

(manuscript in preparation) but I would like to list some of the predictions from this idea which can be tested experimentally. The following predictions assume that all the myosin filaments contain a core protein and are not hollow. The numerical values given obviously depend critically on the exact values taken for the filament diameters (which are only known approximately) and may need to be modified when even more accurate values are available. (i) All myosin-containing filaments of the same backbone diameter will have approximately the same proportion of myosin to core protein by volume and also by weight if the core proteins have the same density. (ii) In general the cores of myosin-containing filaments will have a diameter (or thickness) equal to the total diameter (or thickness) of the filaments less about 70 Å. (iii) In particular the core of the myosin-containing filaments in VStM would have a diameter of about 70 Å and would contain about 10–15% by weight of the total mass of the filaments. This assumes that the density of the core protein is the same as that of the myosin rods and that the projections contain 60% of the mass of the myosin molecules. A model with two molecules per 144 Å repeat would have about 40% of the filament mass in proteins other than myosin. (iv) In insect flight muscle approximately 20–25% of the mass of the myosin filament would be in the core (using the same assumptions) and the diameter of the core would be about 130 Å. Reedy's model with four myosin heads per 146 Å repeat would have about 40–45% of the filament mass in the form of proteins other than myosin. (v) In VSmM the ribbons which have a core of thickness about 75 Å (J. V. Small, and J. M. Squire, in preparation) would have a total ribbon thickness of about 145 Å and the core would account for approximately 25–30% of the total mass of the ribbons. (vi) The structure of the myosin layer will be different in the regions of cylindrical filaments corresponding to the bare zone. On the basis of the type of molecular packing scheme proposed by Huxley²⁰ in which myosin molecules pack antiparallel in the bare zone, the type of packing scheme proposed recently³⁸ from studies of myosin assembly may well occur if modified to fit the filament lattice described here. The thickness (35 Å) of the layer of myosin rods in regions other than the bare zone is that required to close pack the rod portions of about two or three molecules, and it is possible to generate very simple packing arrangements for the myosin rods in these layers which will produce the required surface lattices. Details of these will be given elsewhere. The lattices which have been drawn in Figs. 1a, 2, 3 and 6 are not all identical, but the very small differences in packing which would change one lattice to another are attributable to the small differences in chemical composition of the rod portions of the different myosin molecules involved. It is also possible that the packing of the core protein(s) affects the precise ordering of the myosin molecules on the surfaces of the filaments. (vii) One further implication of the model is that the core protein(s) and not the

myosin in a filament will determine the precise shape and size of that filament. On this basis the vernier length-determining mechanism proposed by Huxley and Brown⁸ would require the presence of at least two core proteins in the thick filaments of VStM and IFM.

Many experiments come to mind which would test the ideas presented here. The extraction and identification of core proteins, together with an estimation of their quantity, is one obvious type of experiment. Electron microscopy could be used to analyse further the cross-bridge arrangements on the myosin-containing filaments of different striated muscles. If it turns out that there is a structure common to all myosin-containing filaments it will be an open question whether or not there is any relationship between that structure and the function of the different muscles in which the filaments occur. One might suggest that the overall shape and size of the filament affect the speed and function of the different muscles but that the precise cross-bridge lattice does not. It would still remain to be answered whether this form of lattice would be critical for an efficient acto-myosin interaction during contraction of a muscle and therefore for an efficient sliding filament mechanism.

I thank Professor J. Lowy for permission to use the micrograph shown in Fig. 7 and him, Professor J. Hanson, Dr J. Haselgrove, Dr J. V. Small and Dr P. J. Vibert for comments on the text.

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Cordilleran Tectonic Transitions and Motion of the North American Plate

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This article examines the relationship between tectonic events in western North America and in the North Atlantic Ocean.

I WISH to propose that there is a correlation between the timing of principal tectonic events in western North America since late Jurassic time and submarine data from the North Atlantic Ocean. The correlation is particularly striking if inferences about relative plate motions from Atlantic magnetics¹ are combined with inferences about absolute plate motions²⁻⁴.

An analysis of the timing of tectonic events in western North America since late Jurassic suggests that important periods of tectonic reorganization (tectonic transitions) took place at about 80 m.y. BP and at about 40 m.y. BP. The data strongly indicate that the tectonic transitions were felt from Alaska to northwestern South America and that they represent fundamental turning points in the tectonic evolution of the Cordillera. The tectonic transitions are best described as boundaries of duration about 5 to 10 m.y. which separate longer periods of up to 60 m.y. The transition periods mark reorganizations of tectonic behaviour, whereas the longer periods separated by transitions are more internally consistent.

The 80 m.y. Transition

The principal significance of the 80 m.y. transition is that it marks the boundary in time and space between classic Laramide orogeny⁵ and Nevadian-Sevier orogeny⁶. The transition extends, however, from Alaska to northern South America, far from type areas in the western United States.

Features associated with the transition include the end of

major plutonism in the Sierra Nevada of California⁷ and the termination of Franciscan deposition^{8,9}. In southern California an important unconformity at the base of the Asuncion Formation at about 80 m.y. places a roof on major metamorphism and plutonism^{10,11}. In southern Alaska, plutonism was initiated near 80 m.y. after a pause of more than 50 m.y. (ref. 12), and deformation of the slate-greywacke belt in the Kodiak-Kenai-Chugach mountains around the Gulf of Alaska began¹³⁻¹⁵. There is some evidence from northwestern Canada that right strike-slip on the Tintina fault diminished after 80 m.y. (ref. 16), but through going right strike-slip on the Chatham fault in southeastern Alaska may have started about this time¹⁷. In the Canadian Cordillera the transition marks the boundary in space and time between late Jurassic to early upper Cretaceous Columbian orogeny¹⁸ and Laramide orogeny¹⁸. There is a low in the frequency of radiometric age determinations from western Canada at about 80 m.y. just before a compound peak extending to 40 m.y. (ref. 19), and there is a marked increase in amount of detritus shed from the Cordillera eastward over the plains from about 80 m.y. to late Palaeocene²⁰. Most deformation to the west of the Sevier orogenic belt of eastern Nevada and western Utah, and within this belt, ceased at about this time⁶ whereas Laramide orogeny^{21,22}, accompanied by a distinct increase in plutonism, broke across the Cordilleran foreland soon afterwards. From the southwestern United States to southern Mexico, widespread carbonate shelf conditions in the Mexican geosyncline terminated near 80 m.y. and were followed by detrital wedges, plutonism, and major compressive deformation^{23,24}. In the Greater Antilles, arc volcanism, plutonism, emplacement of ultramafics and compressive deformation seem to be bracketed by Turonian (about 85 m.y.) and upper Eocene (about 40 m.y.)²⁵⁻²⁸. Deformation and metamorphism in the Venezuelan Coast Ranges ceased in late Cretaceous time and were replaced by a distinctly different tectonic behaviour involving considerable oblique right strike-slip and related thrusting²⁹.

Widespread deformation and plutonism initiated or intensified near 80 m.y. in the northwestern Andes of South America^{30,31}.

Transition at 40 m.y.

The principal significance of the 40 m.y. transition is that it marks the end of Laramide compressive deformation in the western United States³²; the transition can, however, be tracked from northwestern Canada south to the Isthmus of Tehuantepec in southern Mexico, and in the Greater Antilles. In Western Canada, with the exception of the Cascade-southeastern British Columbia region, plutonism and volcanism also almost cease at 40 m.y. (ref. 19), and in the western United States there is a pause in igneous activity³³. After this pause there was a widespread increase of ignimbrite eruption in the Rocky Mountains and Great Basin^{33,34}. In the Greater Antilles, plutonism, volcanism, emplacement of ultramafics, and compressive deformation apparently finished near 40 m.y., but the Lesser Antilles appear to have initiated activity at about this time²⁵. Even in California, compressive deformation involving thrusting of Great Valley rocks over Franciscan rocks appears to have ceased near late Eocene time³⁵.

Several regions are not noticeably affected by the 40 m.y. transition. Southern Alaska, west of the Fairweather fault system, seems to have experienced plutonism from about 80 m.y. to 15 m.y., and compressive deformation from about 80 m.y. to 35 m.y., then again from about 20 m.y. to the present¹⁵. The northwestern Andes are not strongly effected by the 40 m.y. transition, and tectonism and plutonism, which accelerated after 80 m.y., have continued intermittently to the present^{30,31}. Tectonics from Alaska to South America after 40 m.y. has been very variable from region to region, but the timing of volcanism, plutonism, compressive deformation, rifting, and strike-slip faulting fits well with predictions from submarine magnetics in the northeastern Pacific Ocean³⁶. A mid-Tertiary tectonic transition, reported to have taken place between about 30 and 9 m.y. from region to region around the Pacific margin³⁷, is characterized by renewal of strike-slip and related compressive deformation on the San Andreas fault of California, block faulting in the Great Basin, volcanism and plutonism in the Aleutians, southern Alaska, the Cascades, and in Mexico and Central America^{36,38-41}.

Transitions and Plate Motion

The fact that both the 80 and 40 m.y. tectonic transitions cut through the whole array of data for western North America strongly suggests that these important turning points in Cordilleran tectonic evolution are the result of fundamental reorganizations in plate motions.

Changes in relative plate motions in the northeastern Pacific are indicated at about 55 m.y. and 7 m.y. by departures from parallelism of adjacent magnetic anomalies at these times⁴². Atwater^{36,43} has also suggested that changes in plate motions took place at about 80 m.y. and about 15 m.y. as well. There is apparently nothing in the geometry of magnetic stripes in the northeastern Pacific Ocean which would suggest that anything unusual, such as the end of the Laramide orogeny and related deformation, occurred between, say, 50 and 40 m.y. on the adjacent North American continent. This suggests that we are looking in the wrong ocean.

Turning to the North Atlantic for relative motions of the North America plate, Pitman and Talwani¹ suggest that changes in relative plate motion occurred at about 80, 63, 53, 38, and 9 m.y. (their work was based on the analysis of magnetic anomalies). Although spreading between North America and Africa may have started after 180 m.y. and continued to the present, it is important to realize that spreading between North America and Eurasia apparently commenced at about 80 m.y. This is suggested by the fact that north of the Azores, the oldest anomalies and extrapolated time lines adjacent to present continental shelves are 80 m.y. old or less. This would

suggest that spreading broke into the North Atlantic north of the Azores at about 80 m.y. and that spreading subsequently moved into the Arctic, thus separating the two continents. A major reorganization of plates at about 80 m.y. is indicated. Also, Pitman and Talwani¹ suggest that spreading between North America and Europe almost ceased between 38 and 9 m.y. The evidence for this is the close proximity of anomalies 15 (38 m.y.) and 5 (9 m.y.). From the analysis of sea-floor spreading in the North Atlantic, the 80 and 40 m.y. time lines seem important because the start of spreading between North America and Europe at 80 m.y. and its almost complete termination near 40 m.y. bracket the Laramide orogeny and related deformation in western North America. This suggests that both the 80 m.y. and 40 m.y. tectonic transitions, and the tectonic responses in the western Cordillera before, between, and after these transitions, were strongly influenced by motions of the North America plate; these were reflected in the relative spreading history recorded on the floor of the North Atlantic.

Absolute Plate Motion

If the proposal mentioned previously is valid, there is a strong incentive to determine absolute motions of the North America plate during the past 180 m.y. The only hypothesis which would permit analysis of absolute motion is that of Morgan²⁻⁴ who has resurrected a long dormant notion of Wilson⁴⁴ that aseismic ridges and seamount chains on the ocean floors are generated by plate motion over mantle "hot spots" and mark dead reckoning tracks of absolute plate motion. A similar idea has been used by Dietz and Holden⁴⁵. Seamount chains in the North Atlantic are of particular concern because they might provide a fix on the trajectory of the North America plate. The principal seamount chain west of the Mid-Atlantic ridge is the New England (or Kelvin) chain which extends from off Cape Cod in a southeasterly direction towards the Corner Rise near 34° N, 51° W. The only "hot spot", or convection plume²⁻⁴, apparently capable of generating the New England-Corner Rise chain is the Azores³. Dating of the time of formation of the New England-Corner Rise chain would perhaps give the direction of absolute motion of North America during that time.

The White Mountain magma series of New England is a complex of granitic intrusions, rhyolite dikes, calderas and volcanics which apparently initiated activity about 180 m.y. ago⁴⁶. The complex cannot be related to subduction at a continental margin and seems to have more to do with the opening of the Atlantic Ocean⁴⁷. Morgan³ has suggested that the New England seamount chain might anchor on the White Mountain magma series and that both may have been produced by the Azores "hot spot". This suggests that the seamount chain was generated by a northwesterly motion of the North America plate over the Azores "hot spot" as the plate moved away from a proto-Atlantic ridge after 180 m.y. BP. The age of the southeast end of the chain near the Corner Rise is apparently 80 m.y. old or less, because anomaly 32 is close by¹ and drilling at Joides Site 10 (ref. 48) was into Campanian sediments just south of the southeast end of the chain. Because the Azores lie northeast of the Corner Rise, motion of the North America plate must have abruptly changed from northwesterly to southwesterly after the "hot spot" constructed the Corner Rise at or after 80 m.y. The change in plate motions suggested by initiation of spreading between North America and Europe at about 80 m.y. may correspond to the "elbow" at the Corner Rise. No clear seamount chain extends from the Corner Rise to the Azores, but the Corner Rise heads in that direction. Continuity may have been broken by proximity of the "hot spot" to the spreading ridge axis and associated transform faults⁴⁴. Relative motion data¹ suggest that North America presumably drove southwesterly to south-southwesterly after 80 m.y., then slowed down between 40 m.y. and 9 m.y. Thus the northwesterly rotation between 180 and 80 m.y. brackets Nevadan-Sevier-Columbian orogeny, and

the southwesterly rotation between 80 and 400 m.y. brackets the Laramide orogeny.

Conclusions

I propose that after the North America plate broke away from the Africa plate between 180 and 150 m.y. it migrated northwesterly over the Azores "hot spot" until about 80 m.y. (Fig. 1a). During this time the leading edge of North America failed over an overridden and entrained Benioff zone producing the Nevadan-Sevier-Columbian orogeny. A mid to late Jurassic age for initiation of actual separation⁴⁹ would fit well with the initiation of detrital wedges shed eastward from the Cordillera starting in upper Jurassic time^{6,20}. It seems likely

that this northwesterly rotation drove the Alaska-Kolyma⁵⁰ arm of the North America plate into the trailing edge of Eurasia in upper Jurassic to lower Cretaceous time⁵¹ to produce the Verhoyansk Mountains and several intraplate adjustments in Alaska. Some transform rifting between North America and Eurasia in the Arctic is indicated. Once North America and Eurasia had locked and the Alaska-Kolyma peninsula between them had consolidated, North America began separating from Eurasia north of the Azores at about 80 m.y. and rotated southwesterly about a pole near northeastern Alaska. As spreading later broke into the Arctic, the rotation became more complex and eventually reached its present position at the head of rifting in Siberia. The change

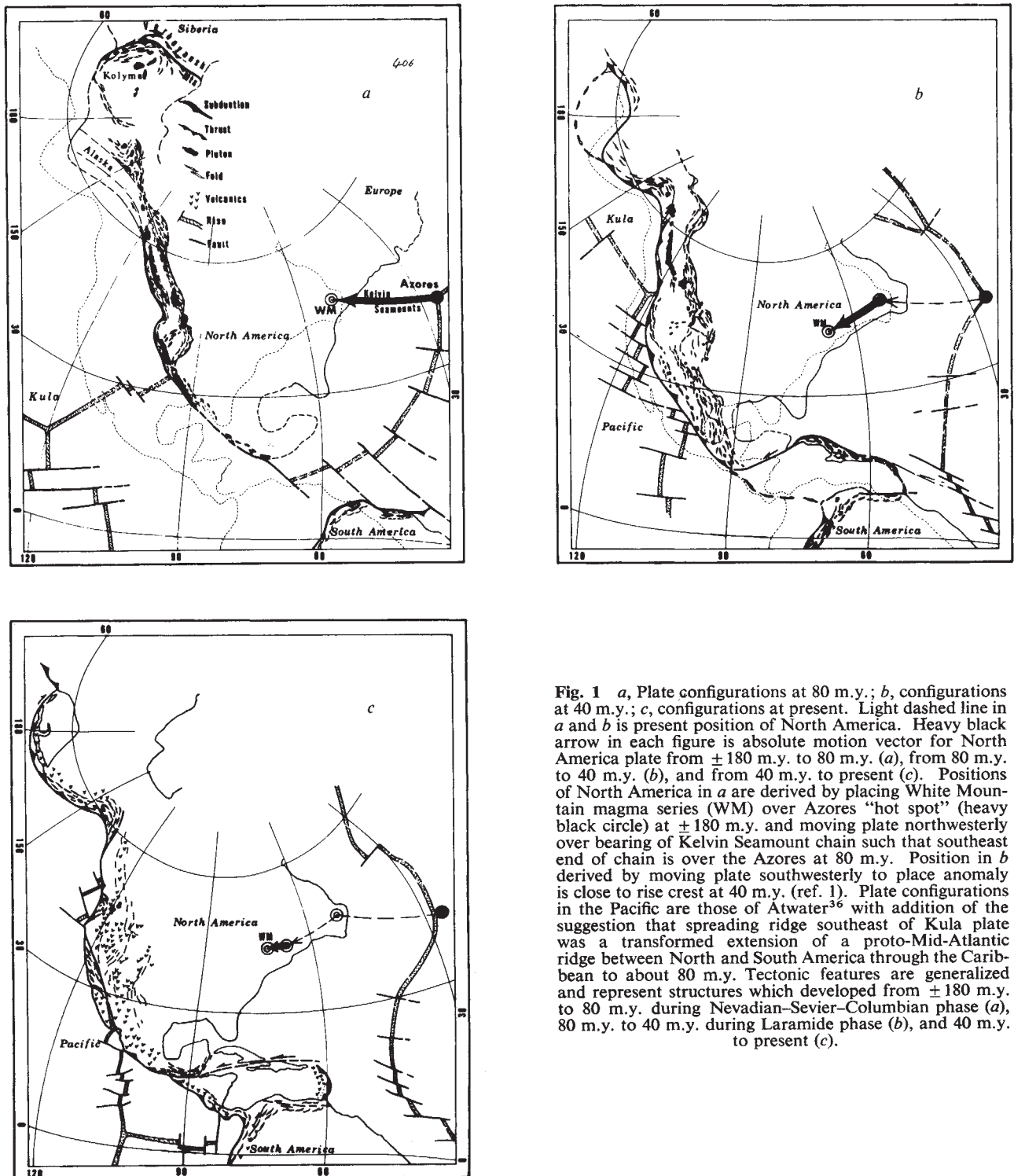


Fig. 1 a, Plate configurations at 80 m.y.; b, configurations at 40 m.y.; c, configurations at present. Light dashed line in a and b is present position of North America. Heavy black arrow in each figure is absolute motion vector for North America plate from ± 180 m.y. to 80 m.y. (a), from 80 m.y. to 40 m.y. (b), and from 40 m.y. to present (c). Positions of North America in a are derived by placing White Mountain magma series (WM) over Azores "hot spot" (heavy black circle) at ± 180 m.y. and moving plate northwesterly over bearing of Kelvin Seamount chain such that southeast end of chain is over the Azores at 80 m.y. Position in b derived by moving plate southwesterly to place anomaly is close to rise crest at 40 m.y. (ref. 1). Plate configurations in the Pacific are those of Atwater³⁶ with addition of the suggestion that spreading ridge southeast of Kula plate was a transformed extension of a proto-Mid-Atlantic ridge between North and South America through the Caribbean to about 80 m.y. Tectonic features are generalized and represent structures which developed from ± 180 m.y. to 80 m.y. during Nevadan-Sevier-Columbian phase (a), 80 m.y. to 40 m.y. during Laramide phase (b), and 40 m.y. to present (c).

in motion at about 80 m.y. apparently caused the 80 m.y. transition, and the new southwesterly motion from 80 to 40 m.y. initiated widespread failure of the Cordilleran foreland to produce the Laramide orogeny (Fig. 1b). A slowing of that motion after 40 m.y. ended that orogeny. I suggest that the 30° bend in the strike-slip faults of Alaska⁵² can almost be eliminated by a rotation of the Corner Rise to the Azores about a pole in northeastern Alaska, thus explaining the Alaska oroclinal^{15, 52-54}. Other features, such as subduction of Atlantic lithosphere by the Greater Antilles between 80 and 40 m.y., are perhaps also explained by North America plate motion.

Since 40 m.y. (Fig. 1c) North America has moved in more of a southwest by west direction towards the East Pacific Rise; eventual contact with the rise off southern California in mid-Tertiary time³⁶ and possible changes in Pacific plate motions near 25 m.y. (ref. 41) explain much of the tectonic history after 40 m.y. Motion of North America and South America past an almost stationary Caribbean plate was probably slightly convergent and explains many features on the margins of the Caribbean such as oblique right strike-slip in northern Venezuela²⁹, compressive and left strike-slip deformation in Chiapas, Mexico and Guatemala^{55, 56}, disruption of the Greater Antilles and Central America along the Bartlett-Motagua fault zones⁵⁷, volcanism in the Lesser Antilles and present seismicity⁵⁸.

The close correspondence of phases of major compressive deformation during Nevadan-Sevier-Columbian and Laramide Cordilleran tectogenesis with timing of North America plate motions suggests to me that compressive tectonics in western North America are the result of abortive west-directed efforts at subduction of continental lithosphere on the leading edge of an actively driving continental plate. This deformation was perhaps facilitated by softening of the plate margin (up to 1,000 km wide) by complex plutonic patterns associated with overridden, entrained and sometimes double⁵⁹ subduction zones. This suggests that active plate motion somehow transmits compressive stress through the plate and that Cordilleran tectonic styles such as those in the Rocky Mountain fold and thrust belt are due to massive westward cratonic underthrusting as visualized by Misch^{60, 61}. By contrast, it would appear that active driving and underthrusting of oceanic lithosphere are easily accomplished and little stress is transmitted across subduction zones from the subducting oceanic lithosphere to the upper plate much beyond the arc. If North America had remained fixed during Mesozoic and early Tertiary time the Cordilleran orogen would more likely have resembled present day western Pacific arcs and margins where active subduction-related tectonics occurs in relatively narrow belts compared with Cordilleran styles. Thus if an Andean-Cordilleran type orogen exists⁶² it is confined to leading edges of actively driving continental plates, and one can conclude that several other combinations of absolute motions are capable of producing as many other orogenic types.

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LETTERS TO NATURE

PHYSICAL SCIENCES

New Point γ -ray Source Lib γ -1: Evidence for Time Variation and Possible Identification with PKS 1514-24

DURING the past 2 yr evidence has been presented for the existence of four point sources of high energy γ -radiation ($E_\gamma > 50$ MeV). Three were found by the Case-Melbourne (C-M) collaboration using high altitude balloon flights from Australia (Fig. 1) and two of these sources, G γ 2+3 and G γ 341+1, coincided closely with known high energy X-ray sources^{1,2}. A group from the Moscow Physical Engineering Institute detected a source with a counter telescope on Cosmos 251³. Three aspects of the Russian source are particularly noteworthy: (1) the source position, $54^\circ < \alpha < 75^\circ$, $+4^\circ < \delta < +9^\circ$, contains the unusual Seyfert galaxy 3C 120⁴ which varies at both radio^{5,6} and optical⁷ frequencies; (2) the γ -ray flux is $F_\gamma = 5 \times 10^{-4}$ γ s cm^{-2} s^{-1} ($E_\gamma > 100$ MeV), a factor of 30 greater than any of the C-M sources; and (3) most of the detected γ -ray flux must have been due to photons above 500 MeV, because inserted between the counters of the Cosmos counter

telescope were 3.7 radiation lengths of lead in which multiple scattering and energy loss greatly reduce the efficiency between 100 and 500 MeV. The Russian group's claim for time variability of 3C 120 as a γ -ray source, because it was not observed on earlier surveys, is not valid. The surveys in this energy range which were cited (refs. 8 and 9) did not cover this region of the sky. The OSO-3 detector (ref. 10), which did cover this area, has a sensitivity to point sources which is about the same as the reported flux from 3C 120.

In this article we report a further point γ -ray source Lib γ -1 detected on the balloon flight of November 26, 1969, which was described earlier (flight III of ref. 2). The signal-to-noise ratio is 6σ for $E_\gamma > 100$ MeV, better than for any of the previous sources and furnishing further confirmation for the existence of this type of object. No excess was seen from this direction on an earlier flight, so for the first time we have direct experimental evidence for the time variation of a γ -ray source. The angular resolution circle in the direction of Lib γ -1 includes PKS 1514-24¹¹ which is also an optical variable^{12,13}.

The method of analysis is similar to that used previously^{1,2} except that, after the approximate source position was ascertained from the search programme which had rectangular bins of $\Delta\alpha = 4^\circ$, $\Delta\sin\delta = 0.08$, a series of concentric annular rings of equal area was drawn about the preliminary location of the source: $\alpha = 227^\circ$, $\delta = -22^\circ$. The radius of the first circle was 2.12° with an outer radius of 6° for the eighth annulus. The higher flux from Lib γ -1, combined with the reduced atmospheric background found at the 8.8 GeV geomagnetic cutoff for flight III, made this more detailed analysis possible. In Fig. 2 is shown the number of events for succeeding rings proceeding out from the source position. After the fourth ring, which has a radius of 4.2° , the intensity has decreased to the background level. The number of events for the energy groups—*a*, all energies (the effective threshold is 50 MeV); *b*, $E_\gamma > 100$ MeV; *c*, $E_\gamma > 500$ MeV—are listed in Table 1. For $E_\gamma > 100$ MeV there is an excess of 40 events above the 45 predicted from the background level, a 6.0σ result. The flux for $E_\gamma > 100$ MeV is $F_\gamma = (2.4 \pm 0.6) \times 10^{-5}$ γ s cm^{-2} s^{-1} , greater than for Sgr γ -1, G γ 2+3, or G γ 341+1, but still a factor 20 less than for the Cosmos 251 source.

Our flight II (ref. 2) also had a good exposure to this region but, as can be seen in Fig. 2*d*, no evidence for Lib γ -1 was found on this flight, which was made on February 26, 1969. Thus only an upper limit can be set. With 95% confidence the flux is $F_{95} < 1.4 \times 10^{-5}$ γ s cm^{-2} s^{-1} . The γ -ray flux increased by at least a factor of two over this nine month interval.

It is not surprising to find a time variation in the γ -ray flux from a localized object since a great variety of time variations is seen at lower frequencies. In particular high energy X-ray sources have appeared and disappeared between exposures and have also exhibited flare type of emission with a time scale of a few minutes¹⁴. We have examined our data to see if any variation occurred during the flight itself, but as is shown in Fig. 3, the number of events per half hour interval follows the expected response within the statistical accuracy.

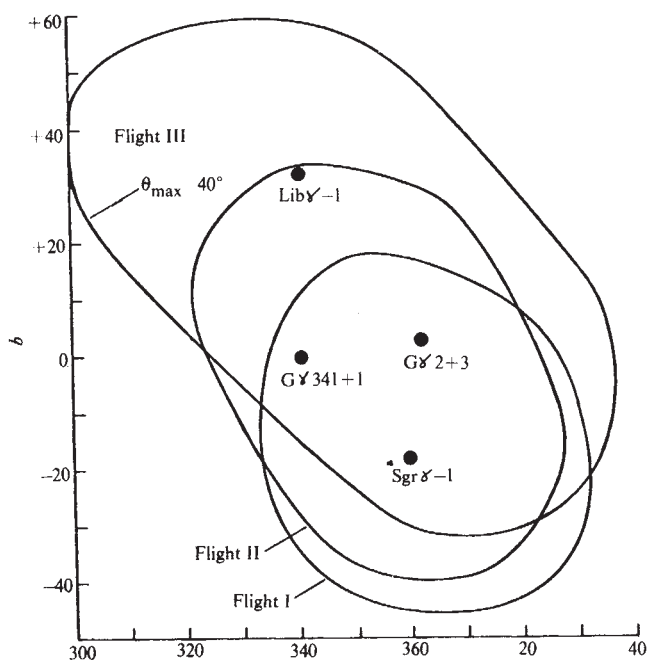


Fig. 1 Sky regions covered by the three flights. Boundaries are shown where the exposure is 50% and events are restricted to zenith angles $0 < 40^\circ$. The black dots represent the positions of the four sources so far observed including the new source Lib γ -1 at $l = 340^\circ$, $b = 30^\circ$.

Table 1 Number of Events detected in Different Energy Groups

Flight date and geomagnetic cutoff	Source	Energy groups	No. x in central area *	Background in equal area N^*	D_x	P_x	Flux γ s cm $^{-2}$ s $^{-1}$
III Nov. 26-27, 1969 2224-0636 UT 8.8 GeV	Lib γ -1	All energy	121	75	6.4×10^{-7}	6.4×10^{-5}	$2.8 \pm 0.8 \times 10^{-5}$
		$E > 100$ MeV	85	45	4×10^{-8}	4×10^{-6}	$2.4 \pm 0.6 \times 10^{-5}$
		$E > 500$ MeV	25	14	5×10^{-3}	0.39	$0.7 \pm 0.4 \times 10^{-5}$
II Feb. 26-27, 1969 1815-0205 UT 4.5 GeV	Lib γ -1	$E > 100$ MeV	60	66	0.82	1.0	95% upper limit 1.5×10^{-5}

D_x is probability of one distribution having number x or greater with mean N .

P_x is probability of one "central" area with number x occurring in an area 100 times the central area—that is, the area observed (assuming Poisson distribution).

* To nearest whole number.

Recently Bond¹⁵ and Biraud¹³ have pointed out that the radio source PKS 1514-24, identified as an E or N galaxy^{11,16}, coincides with the remarkable optically varying object AP Lib, which has been observed since 1935 to vary in magnitude between 14.5 and 16.4. A variation of 0.7 magnitude was observed in this source over one 5 h interval. In a systematic search for coincidences between irregular variable stars and radio sources Biraud added AP Lib and W Com to BL Lac and BW Tau which were already known. Our preliminary position for Lib γ -1 is $\alpha = 227^\circ \pm 2^\circ$, $\delta = -22^\circ \pm 2^\circ$; 2.7° of arc from the optical and radio position of PKS 1514-24, $\alpha = 228.7^\circ$, $\delta = -24.2^\circ$ and within our angular resolution circle.

It is remarkable that two of the three γ -ray sources which are not correlated with X-ray sources should coincide with two of the four objects listed by Biraud. An earlier γ -ray survey⁹ covered BL Lac and W Com but they should be observed again for as long an exposure as possible in view of the time varying nature of Lib γ -1.

Shklovsky¹⁷ has proposed a theory which explains the radio variations of 3C 120 and predicts surges of γ -rays from it and similar objects such as QSOs and Seyfert galaxies which show time variations at lower frequencies. The γ -rays are generated by bremsstrahlung from the fast electrons which produce the varying radiation at lower frequencies by synchrotron radiation. Shklovsky predicts the surges of γ -rays should last the order of several days or weeks and be separated by intervals the order of a year. Our observation is consistent with this time scale since we could detect no large variation over 6 h for Lib γ -1 but a definite variation over 9 months. If Shklovsky's theory is correct, γ -rays should be observed

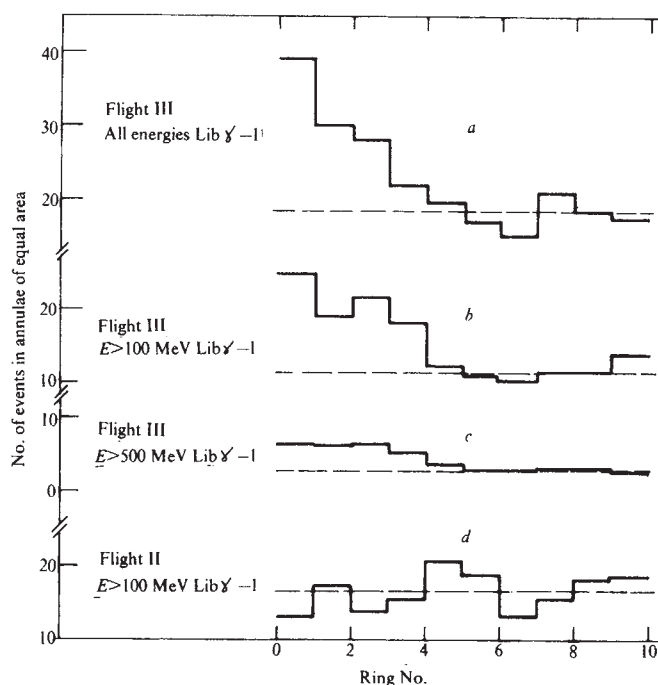


Fig. 2 Number of events plotted for circular annulae about the central position of source Lib γ -1 for flight III and flight II. The radius of ring number 1 is 2.1° and of ring number 8 is 6.0° . The dashed lines correspond to the means for the rings from number 4 to number 8.

Table 2 Comparison of γ -source Positions with Objects listed by Biraud¹³

Possible γ -source	RA	Dec.	Radio and/or optical source	Radio and optical position RA Dec.	Type
Lib γ -1 (ref. 1)	$227^\circ \pm 2^\circ$	$-22^\circ \pm 2^\circ$	AP Lib PKS 1514-24	228.7° -24°	E or N, variable
(ref. 3)	$54^\circ < \alpha < 75^\circ$		BW Tau 3C 120 PKS 0430+5	67.5° $+5^\circ$	Seyfert, strongly variable
	$+4 < \delta < +9$		W Com ON 231	184.5° $+28^\circ$	Variable
			BL Lac VRO 42 22 01	330.0° $+42.0^\circ$	Similar to AP Lib

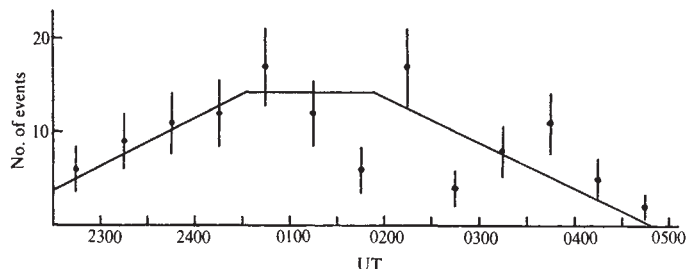


Fig. 3 Number of events per half hour interval received from the source direction during flight III. The full line shows the expected response curve for a constant source.

from a given object only 1–10% of the time and thus supposedly our second period of observation coincided with an outburst. An extended period of observation would then be required to delineate the time history of Lib γ -1. The γ -ray energy spectrum will furnish an additional test of the theory. If the spectrum follows a power law in the 10–100 MeV region, the corresponding electron energy spectrum can be deduced. If, however, it is a pion spectrum with the characteristic peak at 70 MeV (which may be red-shifted if PKS 1514–24 is far away), nucleon–nucleon collisions or nucleon–antinucleon annihilation will be responsible for the γ -radiation and Shklovsky's mechanism will be ruled out.

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Short Term Variability of Pulsations in the X-ray Flux from Cygnus X1

IN a recent sounding rocket flight in the southern hemisphere (launched from Woomera, South Australia, on November 11, 1970) the X-ray source Cygnus X1 was observed by a 1,000 cm² collimated proportional counter sensitive to X-rays in the range 2–14 keV. Each X-ray event was indicated through the telemetry link by a 300 μ s analogue pulse whose height was proportional to the incoming photon energy. This method of data transmission allows good timing accuracy together with a reasonable spectral resolution.

The data were folded using a technique similar to that used by Boldt *et al.*¹ at a number of trial periods in the range 50 ms to 1.2 s, and a chi-squared statistical test carried out on the resulting light curve to search for the presence of significant periodic variations in the X-ray flux.

The detector observed the source twice during the flight, the two scans being separated by 55 s. During the first scan significant fluctuations were observed at periods of 57.50, 115.24 and 229.74 ms. Table 1 shows the significance of the departure from the expected count rate. P is the probability that such a value of chi-squared would arise from random data with no pulsation present. The smaller the value of P the more significant the fluctuation. Analysis of the second scan revealed no significant pulsation at the $P=0.01$ level at any period in the range tested. The maximum values of chi-squared

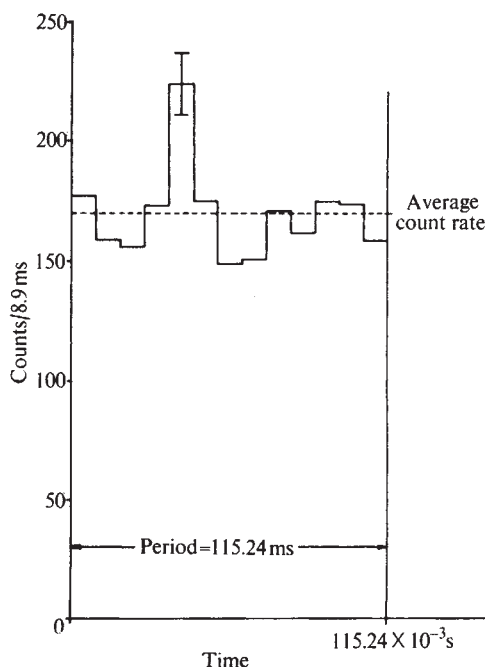


Fig. 1 "Light curve" at 115.24 ms period.

and the periods at which they were observed are also shown in Table 1. We interpret these results as indication of a drop in the fluctuating fraction of the X-ray output of the source in the 55 s separating the two scans. If similar behaviour is present at higher energies it may explain the negative result of Manchada *et al.*².

Table 1 Significance of Departure from Expected Count Rate

Period	Chi-squared (for $\nu=10$)	P
Scan 1		
57.50 ms	29.4	0.001
115.24 ms	24.0	0.01
229.74 ms	23.3	0.01
Scan 2		
55.66 ms	19.5	0.04
113.55 ms	15.5	0.10
231.93 ms	14.4	0.11

The steady flux from Cygnus X1 was the same as measured by previous observers and showed no change between the two scans to within the experimental accuracy of 3%.

Fig. 1 shows the light curve at 115.24 ms, the only light curve in these data that is easily interpreted at the present time. By altering the number of phase bins within the light curve we have estimated the width of the pulse and also the maximum count rate during the pulse. These parameters and estimates of the pulsed energy output of the source are listed in Table 2. As the light curves at the other periods reported here are more complex we defer discussion of their interrelationship to a later communication.

Table 2 Measured Parameters of the 115.24 ms Light Curve

Period	115.24 ms
Pulse width	9.2 ± 0.4 ms
Count rate during pulse	0.64 ± 0.18 photons cm^{-2} s^{-1} in range 2–14 keV
Average flux	1.31 ± 0.05 photons cm^{-2} s^{-1} in range 2–14 keV
Energy in pulse as a fraction of total energy	0.04 ± 0.01
Pulsed energy (with period 115.24 ms) radiated by source, in energy range 2–14 keV (assuming a distance of 1.0 kpc (ref. 5))	1.5×10^{34} erg s^{-1}

The only period detected in this flight that has been previously reported is that of 230 ms. Other observers have detected periods of 290 ms and 1.1 s (ref. 3) and 73 ms (ref. 4), although these were not significant in our data. We note, however, that there are certain harmonic relationships between some of these periodicities.

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Soft X-ray Emission from Galactic Radio Spurs

Shklovsky and Sheffer¹ have recently raised the interesting possibility that the galactic radio spurs could be soft X-ray sources. As radio spurs have often been considered to be very old supernova remnants^{2,3}, and since three old remnants have been found to be soft X-ray sources^{4,5}, this idea has particular merit. Shklovsky and Sheffer point out that the soft X-ray (0.21 to 0.28 keV) map of Bowyer *et al.*⁶ shows enhanced emission in the northern galactic hemisphere in a region coincident with secondary radio ridges of the North Polar Spur⁷ and that this correlation may provide experimental verification of the hypothesis concerning the possible supernova remnant origin of the spurs.

The purpose of this letter is to question the proposed association between radio spurs and soft X-ray emission in general and to take issue with the particular identification of soft X-ray emission observed by Bowyer *et al.* with the North Polar Spur. The main points of our argument are that an independent interpretation of the radio spurs exists⁸ which shows most of these features are not related to SNR. A possible exception is the Cetus Arc⁹, a configuration in the southern galactic hemisphere, which we discuss in depth. We conclude on theoretical grounds that this object is not likely to be an X-ray source. From the observational viewpoint the soft X-ray enhancement observed by Bowyer *et al.* can be interpreted in terms of solar X-rays resonantly scattered in the Earth's atmosphere. Other investigators who have scanned the North Polar Spur and portions of other galactic spurs (in particular the Cetus Arc) at large solar zenith angles have not observed any enhancement at soft X-ray energies.

With regard to possible interpretations of the galactic radio spurs, we call attention to the fact that Mathewson⁸ has been able to explain most of the observed radio features in terms of synchrotron radiation from electrons spiralling along a helical component of the local magnetic field. With this interpretation, he also accounts for the polarization orientation of the non-thermal galactic radio background and the large-scale orientation of the optical polarization vectors of nearby stars.

One feature Mathewson's model is not able to explain is the Cetus Arc. The spectral index of the polarized radiation between 408 and 620 MHz is steeper in this region than in others in the Galaxy¹⁰, and H_α filaments^{11,12}, H_β radiation¹³, and neutral hydrogen (M. P. Hughes, A. R. Thompson and R. S. Colvin, unpublished results) have been detected in the region. It seems reasonable to interpret that this feature is an old and unusually large supernova remnant. If the Cetus Arc is indeed such a remnant, it is useful to estimate whether it is likely to be a soft X-ray emitter. The X-ray emission spectrum of a supernova remnant varies with time as it is strongly influenced by the dynamics of the expansion. Three phases can be distinguished in the expansion of a typical shell^{14,15}.

(1) The free expansion phase, which proceeds up to a time when deceleration effects become important. The diameter at such a time is $D = 4.2 (M/M_\odot)^{1/3} n_H^{-1/3}$ pc, where M is the ejected mass and n_H the unperturbed interstellar number density.

(2) A deceleration phase which is energy-conserving (or adiabatic). During this phase the shell diameter varies as

$$D \approx 25.8 \left(\frac{E_0}{n_H} \right)^{1/5} \left(\frac{t}{10^4 \text{ yr}} \right)^{2/5} \text{ pc} \quad (1)$$

where E_0 is the energy released in the explosion in units of 7.5×10^{50} erg and t is the age in years. In this case the shock velocity is given by

$$v_s \approx 505 \left(\frac{E_0}{n_H} \right)^{1/5} \left(\frac{t}{10^4 \text{ yr}} \right)^{-3/5} \text{ km s}^{-1} \quad (2)$$

Heiles¹⁶ demonstrated that this phase continues only up to a time when radiation losses become important and thereafter

the assumption of energy conservation is no longer valid. Cox¹⁵ has shown that when line emission by heavy elements is included in the radiative losses, the thermal energy content of the whole remnant varies with time as

$$\frac{E}{E_0} = 1 - \left(\frac{t}{t_c}\right)^{17/5} \quad (3)$$

where $t_c = 5.6 \times 10^4 E_0^{4/17} n_H^{-9/17}$ yr. The shell diameter at this time is

$$D_c = 50.8 E_0^{5/17} n_H^{-7/17} \text{ pc} \quad (4)$$

Shortly after the remnant has cooled to a dense shell, it enters the next and final phase.

(3) The momentum-conserving phase in which the radius varies as

$$D \sim \left(M_0 v_0\right)^{1/4} n_H^{-1/4} t^{1/4} \quad (5)$$

where $M_0 v_0$ is the shell momentum at the time equation (5) becomes first applicable. Assuming the final shell momentum is 75% of the momentum at the time the temperature of a mass element drops faster from radiation than from expansion, the shock velocity in this phase is

$$v_s \approx 165 E_0^{16/17} n_H^{-1} \left(\frac{D \text{ pc}}{40}\right)^{-3} \text{ km s}^{-1} \quad (6)$$

During phase 1 and early in phase 2 the X-ray emission is most likely nonthermal in nature and due to the high-energy tail of the same electron distribution responsible for the nonthermal radio emission¹⁷. This is probably the case for Cas A, Tycho's supernova and the Crab Nebula. The spectra have already steepened in the X-ray range due to radiation and expansion losses. Continuous injection of high-energy particles seems indicated over a time scale of hundreds of years. For remnants late in phase 2, the X-ray emission is probably thermal radiation from the shock-heated plasma present in the shell. The temperature behind the shock is, for a monoatomic gas,

$$T = \frac{3\mu}{16k} v_s^2 \quad (7)$$

where μ is the mean mass per particle, k is Boltzmann's constant, and v_s is the shock velocity. Using $\mu = 0.61 m_H$, corresponding to a 10% helium abundance by number, for $E_0 = n_H = 1$ and $t = 10^4$ yr, we see from equations (2) and (7) that $T \sim 3 \times 10^6$ K.

As a tentative test of these ideas, we list in Table 1 the diameters, ages and shock speeds for two well studied remnants, Vela X and the Cygnus Loop, with a comparison between the values obtained from equations (1), (2) and (7) and the observations^{4,5}. For Vela X, it is assumed the age of the pulsar PSR 0833-45¹⁸ to be that of the remnant. For

the Cygnus Loop, the expansion velocity of the filamentary system may not be representative of the shock velocity²² and a new estimate for the parameters has been obtained by scaling the Vela X values to the diameter of the Loop using equation (2).

Although our understanding of supernova remnants is far from complete, the reasonable agreement between theory and experiment for these two remnants lends confidence to attempts to use these models to predict the emission spectrum of the Cetus Arc. To do this, we need an estimate of the linear diameter and expansion velocity of this feature.

Hughes *et al.* (unpublished work) have compared the neutral hydrogen absorption velocities observed in the spectra of extragalactic radio sources in the direction of the Cetus Arc with velocities obtained from optical absorption lines in the spectra of nearby stars with known distances in this region. From this they obtain a distance to the centre of the Arc, assuming spherical symmetry, of $d \leq 170$ pc. The linear diameter, as implied from the radio angular diameter, is then $D \leq 240$ pc. The upper limit for the proper motion of the H_α filaments is $0.5 \text{ arc s yr}^{-1}$ (ref. 11) and this provides an upper limit for the expansion velocity of $v_s < 90 \text{ km s}^{-1}$.

From a comparison of these values with equations (4) and (6), we see that the Cetus Arc ought to be well into the momentum-conserving phase. Cox has computed a number of detailed models for supernova remnants in this phase¹⁵. The model which is most appropriate for comparison with the Cetus Arc is characterized by $v_s = 70 \text{ km s}^{-1}$. For this model, Cox derives a shock temperature of $T \sim 6 \times 10^4$ K. Alternately, using equation (7), we find that for $v_s < 90 \text{ km s}^{-1}$, the corresponding temperature is $T < 9.5 \times 10^4$ K. In either case, the upper limit for the temperature of the Cetus Arc is at least a factor of 40 below that needed in order for this object to be a source of soft X-rays, and almost all the energy is radiated in the form of ultraviolet, visible, and infrared lines.

Direct estimates of conditions in the Cetus Arc can be made by scaling values relevant for Vela X to the 240 pc diameter of the Arc using either equations (1) and (2), relevant for a remnant in phase 2, or equations (5) and (6), relevant for a remnant in phase 3. This approach assumes that the parameters E_0 and n_H are the same in the two objects. In equations (1) and (2), the ratio E_0/n_H enters as the one-fifth power; hence, the effects of any possible differences in these parameters tend to be minimized while the remnant is in phase 2. In equation (6), however, this ratio enters directly, so estimates for v_s while the remnant is in phase 3 are less reliable. In Table 1 we list the values for the age, shock velocity, and shock temperature for the Cetus Arc derived in this manner. With both models, the predicted shock speeds are consistent with the observational upper limit; and in both cases, the temperature is far below that necessary for the object to be a source of soft X-rays.

Considerable soft X-ray data that relate directly to this subject are available. The soft X-ray enhancement detected by Bowyer *et al.*⁶ near the North Polar Spur is best interpreted

Table 1 Observed and Computed Parameters for Old Supernova Remnants

Source	Diameter (pc)	Age (yr)	v_s (km s ⁻¹)	kT (keV)		References
				Observed	Predicted	
Vela X	35	1.13×10^4	600	0.23	0.4	5, 18, 19
Cygnus Loop	41	6.7×10^4	110	0.37	0.014	4, 20, 21
		1.8×10^4 *	440		0.23	
Cetus Arc	240	1.4×10^5 *	30	—	1.3×10^{-3}	11, and Hughes <i>et al.</i> unpublished
		2.7×10^7 †	2		5×10^{-6}	

Note: Velocity in italics is an observational value. For the Cetus Arc the observational upper limit to the velocity is $v_s < 90 \text{ km s}^{-1}$. The corresponding temperature is $kT < 8.0 \times 10^{-3} \text{ keV}$.

* Computed using energy-conserving theory, scaled from Vela X.

† Computed using momentum-conserving theory, scaled from Vela X.

as the result of atmospheric scattering of solar X-rays, because the Sun was close to the horizon at this position in the sky at the time of their flight²³. This effect has been examined in some detail by Tomblin²⁴ and appears to be well understood. Other investigators have scanned portions of galactic radio spurs at soft X-ray energies with more favourable solar zenith angles: Henry *et al.*²⁵ and Palmieri *et al.* (unpublished work) have scanned the North Polar Spur; Bunner *et al.*²⁶, Hayakawa *et al.* (unpublished work) and Baxter *et al.*²⁷ have scanned Loop III²⁸; and Bunner *et al.*^{26,29}, Hayakawa *et al.* (unpublished work) and Baxter *et al.*²⁷ have scanned the Cetus Arc. No soft X-ray enhancements are present in the published data of these investigators near the positions of these features.

We conclude both on theoretical and observational grounds that galactic spurs are not, in general, sources of soft X-ray emission. The Cetus Arc may well be a supernova remnant; if it is, however, it has long ago ceased to be a source of X-ray emission.

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Is Mercury from Hawaiian Volcanoes a Natural Source of Pollution?

THE development of more sensitive techniques¹ for the analysis of mercury has resulted in reports of previously unsuspected amounts of mercury in the environment. There are now several well documented cases of mercury pollution deriving from industrial sources, but sources of other forms of mercury pollution, as in ocean-going fish², for example, are not so well defined³. The widespread distribution of mercury in the environment may, however, be in part a consequence of the physical properties of the element, the manufacture of the element by the smelting of cinnabar, and the fact that certain microorganisms can convert mercury to the gas dimethyl mercury.

Another possible source of atmospheric mercury is vulcanism. A recent review⁵ states that "data on mercury contents of fumaroles are lacking because of the rarity of volcanic eruptions and high-temperature fumaroles and, until recently, the lack of adequate methods of analysis. Hawaiian and Alaskan fumaroles should be studied". We now report that the active vulcanism on the island of Hawaii is producing significant amounts of mercury.

Atmospheric mercury was obtained by drawing 0.2–0.4 m³ of air through 15 × 150 mm U-tubes containing traps of 10 ml. of concentrated H₂SO₄, 1 ml. of concentrated nitric acid and about 500 mg of powdered copper (reagent grade). Variations on this procedure included the use of different glassware and the replacement of copper by silver, gold and other heavy metals.

Field air samples were collected using a portable battery-powered pump (Mine Safety Appliances No. 92–814, Bureau of Mines Approval No. 2F-2004). After sample runs of 30 to 90 min duration, trapping vessels were sealed for analysis at the laboratory in Honolulu. Further samples were collected using Mine Safety Appliances No. 93-494 cellulose filters, which retain particles down to 0.3 µm diameter.

Table 1 Atmospheric Mercury Levels in Selected Hawaiian Sites

Sample site and time	Trap	Volume of air sample (m ³)	Hg µg/m ³
A, Manoa Campus, University of Hawaii, Honolulu (Oahu)			
1700–1900 h, April 18, 1971, temp. 25° C, clear, no wind	(1) Cu/acid	0.50	0.034
	(2) Ag/acid	0.47	0.019
	(3) Cu/acid	0.40	0.065
Average			0.04
B, Ala Moana Beach, Mamala Bay, Honolulu (Oahu)			
1200–1400 h, May 13, 1971, temp. 30° C, clear, mild breeze from north	(1) Cu/acid	0.36	0.60
	(2) Cu/acid	0.36	0.71
	(3) Cu/acid	0.36	1.41
Average			0.91
C, Sulfur Banks, Hawaii Volcanoes, National Park (Hawaii)			
1000–1200 h, April 10, 1971, temp. 20°–22° C, intermittent light rain	(1) Cu/acid	0.36	26.6
	(2) Charcoal	0.34	26.3
	(3) Cu/acid	0.36	17.1
Average			23.3
1100–1300 h, May 8, 1971, temp. 22° C, clear to light rain	(1) Cu/acid	0.15	15.4
	(2) Cu/acid	0.34	27.4
	(3) Cellulose filter	0.34	0.38
	(4) Cellulose filter	0.24	0.50
Average			0.44

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Trap contents were transferred quantitatively to acid-washed flasks containing 100 ml. of concentrated nitric acid and diluted to 200 ml. with distilled water. Portions of 20 ml. were analysed for mercury content by flameless atomic absorption at the 253.65 nm resonance line. We used a Utopia Instruments flameless mercury analysis kit⁶ and a 10 cm absorption cell mounted on the burner of a Beckman 1301 atomic absorption spectrophotometer. Samples were compared with appropriate blanks and standards. The results are summarized in Table 1. The Sulfur Banks within Hawaii Volcanoes National Park is a site where steam and gas are emitted more or less continuously through crevices and cracks which vent into the open air. Control samples were taken in the Manoa Valley which has light traffic and no industry. Tradewinds usually carry clean air masses over the head wall of the valley. A second control site was urban, non-residential, and in proximity to heavy traffic, but also was without industrial development.

Table 2 Boiling Points of Selected Mercury Compounds

Compound	Boiling, sublimation (S) or decomposition (D) point (°C)
Hg	356.58
Hg ₂ Br ₂	S 345
HgBr ₂	322
Hg ₂ Cl ₂	S 400
HgCl ₂	302
Hg ₂ I ₂	S 140, D 290
HgI ₂ (β-form)	354
Hg ₂ O	D 100
HgO	D 500
HgS	S 583.5

The results show that 98% of the mercury issuing from the Hawaiian fumaroles is either in the form of a gas or of particles less than 0.3 μm in diameter. Several mercury compounds as well as the pure metal may become gaseous under the conditions of temperature and pressure found in volcanic fumaroles (Table 2)⁷. The magnitudes of mercury content in these fumaroles' discharges agree quite well with reports from thermal areas in Kamchatka and the Kuriles⁵. The background figures for Honolulu seem high when compared with other reports, but this may simply be because this site is close to a volcano.

The Hawaiian Islands possess industries using mercury. The sugar industry (the state's third largest behind the military and tourism), for example, has used mercury-containing fungicides. Mercury batteries, lamps and switches are used by many individuals and businesses. Military consumption of mercury is not known. In spite of the industrial consumption of mercury in Hawaii, however, it is still reasonable to suspect that the volcanoes supply large quantities of mercury to the environment. During a period of heavy volcanic activity it is not uncommon to have a high atmospheric turbidity extending along the island chain. This haze may be very noticeable in downtown Honolulu on the island of Oahu, more than 200 miles NNW of the source. Since our laboratory has turned its attentions toward mercury studies this phenomenon has not recurred so that we have been unable to verify the efficacy of atmospheric dispersal of mercury from volcanic activity. We are now planning a programme of continued air monitoring for mercury.

In conclusion, we do not wish to discount the mercury contribution of industrial pollution, but only to suggest that in Hawaii, at least, natural as well as human factors may be at work.

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Proposed New Method for Separating Isotopes

RECENTLY¹ a double mercury discharge vessel was used (Fig. 1), in which a large current running in the outer vessel irradiated the small current discharge in the inner vessel with 2537 Å resonance radiation. This irradiation caused the running voltage and electron temperature in the inner discharge to be reduced appreciably over the non-irradiated mode of operation. The explanation offered was that the neutral Hg atoms in the inner vessel absorbed the resonance radiation and entered the 6³P₁ state (see Fig. 2), and from this level the atom was ionized by electron impact. In addition the 6³P₀ and 6³P₂ metastable levels are populated from the 6³P₁ level by electron collisions and by cumulative absorption and radiative decay of higher levels. Because the radiation field is doing approximately half the ionization work, the electron temperature drops correspondingly.

The present communication suggests the possibility that such a double discharge could form the basis of an isotope separator, if the outer, irradiating discharge were run in a pure isotope and the hyperfine components of the resonance line are separate. We show in Fig. 3 the hyperfine components of the Hg 2537 Å line and it is seen that ²⁰⁰Hg and ²⁰²Hg could be separated by this method. In this situation only the isotope in the inner discharge which corresponds to that in the outer will be excited, and thus the Hg ions which are accelerated to the discharge wall by the radial electric field will be predominantly of one isotope. The details of the collection of ions at the wall are not considered here.

It is interesting to note that this method differs from other methods in current use in that it does not depend directly on

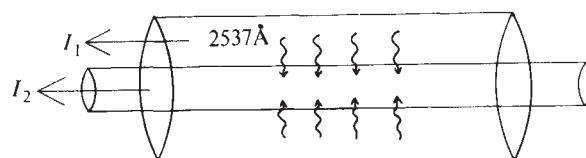


Fig. 1 Optically pumped discharge tube. For use as an isotope separator the outer tube would contain a pure isotope and the inner tube the mixture to be separated. $I_1 \gg I_2$. Radiation wavelength for Hg.

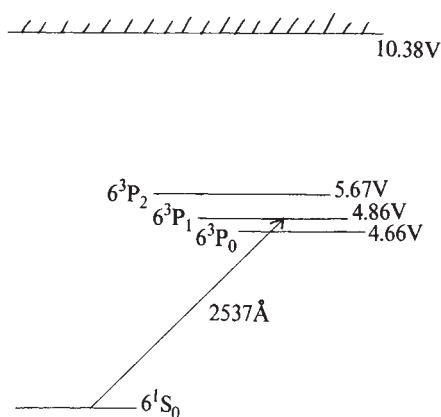


Fig. 2 Simplified Hg energy level diagram.

the isotope mass ratios, which for heavy elements are almost unity.

In the case of Hg isotope separation the inner discharge changes to the radiation-assisted mode only for Hg gas pressures greater than 10 mtorr¹. At these higher pressures the ion on its way to the wall will suffer charge exchange collisions with Hg atoms of all isotopes and so the ion wall current will not be of the pure isotope. In the case of Hg, further experiments in our laboratory have shown that the addition of a few mtorr¹ of N₂ to the Hg facilitates the transition to the radiation-assisted mode, at only 1 mtorr of Hg, where the charge exchange mean free path for Hg⁺-Hg collisions is fairly long. It is believed that this improvement is caused by collisions between Hg 6^3P_1 atoms and N₂ in the $v=0$ vibration state, giving Hg 6^3P_0 and N₂ ($v=1$), (ref. 2), and because the Hg 6^3P_0 metastable state has a much longer lifetime than that of Hg 6^3P_1 , the electron impact ionization makes more efficient use of the radiation assistance. If this is the explanation, then CO should be even more efficient an additive than N₂ (ref. 2), because the quenching cross-section of CO for $6^3P_1 \rightarrow 6^3P_0$ is much greater than that of N₂. Such high ionization potential additives as N₂ and CO result in Hg being the predominant ion even up to molecular gas pressures considerably greater than the Hg pressure.

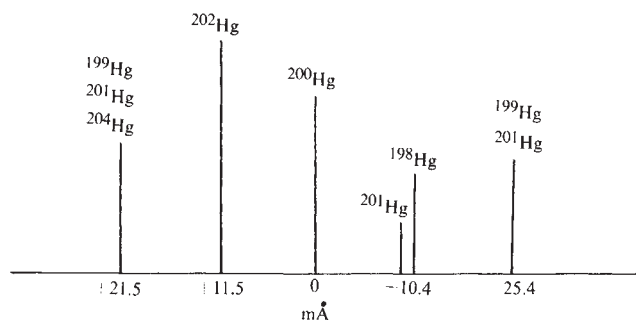


Fig. 3 Hyperfine structure of the 2537 Å resonance line.

Extension of this method to other elements is dependent on the fulfilment of several criteria, among which are that, first, the hyperfine components must not overlap and, second, that the mean free path for radiation absorption be equal to or less than the mean free path for charge exchange.

This latter criterion is probably not satisfied by uranium, although its spectroscopic properties are poorly known³.

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On the Formation of the Solar System

THE object of this article is to point out the significance of some properties of Jupiter (the most massive planet in the solar system) for the problem of the origin of the solar system. The mass of Jupiter is 0.001 solar masses, and its orbital eccentricity is 0.05. The following discussion shows that these properties of Jupiter are *primaevial*, and that they have not been affected by the evolution of the solar system.

I shall start by considering what would happen to the planetary system if the mass of Jupiter and/or the eccentricity of its orbit around the Sun increased. Shelus and I¹ and Shelus (unpublished) have already tried to answer this in a study of the elliptical restricted problem of three bodies. Periodic orbits for a body of infinitesimal mass in the presence of two massive objects, moving around each other in eccentric orbits, were computed. The mass ratios for the two primaries used in this study were 1,000, 100, 10, 5, and 1. The periodic orbits were tested for stability under the framework of a linear stability analysis. From these computations, it is clear that increasing the mass of Jupiter and/or increasing its orbital eccentricity makes the orbits of the "small" planets, such as the Earth, unstable. A small planet, when put in one of the unstable orbits, will tend to collide with one of the primaries, or it will be thrown out of the system. Some stable periodic orbits exist, but they are located either very close to one of the two primaries or very far away from both of them.

If Jupiter had originally possessed 40 or 50 times its present mass, it would have made the planetary system unstable over a period much shorter than 4.5×10^9 yr. The planetary system would also have become unstable if Jupiter's orbital eccentricity were as small as 0.5. It should be mentioned that the increase of the eccentricity tends to destroy the stability of the orbits of the small planets more effectively than does the increase of the mass of Jupiter. Thus the fact that the planetary system has survived for a period of 4.5×10^9 yr leads us to the conclusion that the above mentioned properties of the most massive planet (Jupiter) are original properties of the system. If they are original, then any satisfactory theory of the formation of the solar system must take them into account.

The above discussion is obviously relevant to the problem of the existence of other planetary systems in the Galaxy. It is clear that double stars, which normally have mass ratios in the range 1 to 10 and eccentricities as high as 0.9, cannot possess planetary systems such as our own. Further, double stars, such as Lalande 21185, in which one of the components is a low mass "dark" star, may not possess planetary systems similar to our own, for their mass ratios are in the range 10 to 50, and the eccentricities in the range 0.3 to 0.9. The number

of multiple stars in the Galaxy is very high, and at least 60% of all stars in the solar neighbourhood are found in double systems². As the formation of double stars seems to be favoured, the occurrence of planetary systems cannot be a universal phenomenon. We may conclude that the total number of planetary systems in the Galaxy is probably much smaller than that estimated by some workers³.

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Dust transported in the North-east and South-east Trade Winds in the Atlantic Ocean

THE dust in the atmosphere over the oceans can yield an insight into the processes of deep-sea sedimentation and into certain aspects of oceanic pollution¹⁻⁴. The importance of wind transport for the introduction of land-derived material to the oceans varies from one area to another, and the highest reported content of dust is found in the north-east trade wind system over the North Atlantic Ocean⁵, but there seem to be no data on the dust loadings in the south-east trades of the Atlantic. To establish the concentrations and kind of dust in this important wind system, collections were made during July and August 1970 in both the SE and NE trades of the Atlantic Ocean between about 16° S and 23° N.

The dusts were collected on nylon meshes, and the volume of wind passing through the meshes was calculated using a calibrated anemometer. Two meshes, each 1 m², were exposed for each collection and the weights of dust collected were corrected for the collection efficiency of the meshes, which was assumed to be 50% (ref. 1). Samples with a prefix SH were

collected in the SE trades off the coast of St Helena from a small fishing boat; those with a prefix GH were collected on the RMMV Good Hope Castle. The ship's track, collection areas and dust loadings are shown in Fig. 1, and collection details are given in Table 1.

Dusts collected in the SE trades between about 16° S and the equator have an average concentration of 0.55 $\mu\text{g m}^{-3}$ of air, and are predominantly brown or grey in colour. With the exception of dust GH3, which contained some shipboard contaminants, the dust loadings are quite similar and have only a relatively small range of concentration (0.33–0.70 $\mu\text{g m}^{-3}$ of air). Sample GH4 was collected in variable winds of the intertropical convergence zone, and has a similar dust loading to the SE trades. This sample, however, has a distinct red-brown colour, suggesting an origin for much of the dust in the deserts of West Africa. There was a dramatic change in dust loadings when the ship entered the NE trades; the red-brown dusts have an average concentration of 24.5 $\mu\text{g m}^{-3}$ of air.

Table 2 Particle Size Analyses of the Dusts *

Sample	Particle size class					
	<2 μm	2 to 4 μm	4 to 8 μm	8 to 16 μm	16 to 32 μm	> 32 μm
Average of Dusts SH1 to SH5	74	14	9.0	2.0	1.0	Trace
GH1	60	15	13	6.7	3.2	2.1
GH2	50	20	18	7.0	3.0	2.0
GH3	60	20	13	3.0	2.5	1.5
GH4	91	4.6	2.5	1.7	0.2	Trace
GH5	78	12	6.7	2.2	0.8	0.3
GH6	84	6.8	4.8	2.6	1.2	0.6
M1†	79	13.5	6.5	1.0	Trace	Trace

* Particle size determinations were made by a microscopic counting technique, and the results are given as the percentage number of particles in each size class. The analyst was L. R. Johnson.

† M1 dust collected off West African coast between 17° 39' N, 16° 36' W and 18° 55' N, 17° 22' W—see ref. 5 for collection details. The particle size analysis given in ref. 5 was on a weight percentage basis and differs from that given here which is based on percentage number of particles.

The particle size analyses of the dusts are given in Table 2. Dusts collected to the south of St Helena have only a trace amount of material in the size class > 32 μm . By contrast, dusts GH1, GH2 and GH3 contain more material both in the > 32 μm and the 8 to 16 μm size range; this is probably the

Table 1 Dust Collection Details

Sample	Location *	Wind system	Colour of dust	Dust loading ($\mu\text{g m}^{-3}$ of air)	Remarks
SH1	Off the coast of St Helena	SE trades	Light brown	0.78	Winds SE to SSE
SH2	Island, position 15° 54' S to 16° 01' S	SE trades	Brown-grey	0.33	
SH3	and 05° 37' W to 05° 46' W	SE trades	Brown-grey	0.51	
SH4		SE trades	Light brown	0.54	
SH5		SE trades	Brown	0.37	
GH1	13° 19' S, 08° 41' W	SE trades	Light grey	0.44	Wind SE
	08° 16' S, 14° 36' W				
GH2	08° 16' S, 14° 36' W				
	06° 08' S, 14° 42' W	SE trades	Grey	0.51	Wind SE. Some contamination from ship smoke; dust loadings therefore represent upper limits.
GH3	06° 08' S, 14° 42' W				
	00° 00' S, 16° 00' W				
GH4	00° 00' S, 16° 00' W	Doldrums	Grey-black	0.93	Winds variable
	12° 27' N, 17° 28' W				
GH5	12° 27' N, 17° 28' W				
	20° 20' N, 17° 43' W	NE trades	Red-brown	23	Winds NNE
GH6	20° 20' N, 17° 43' W				
	23° 09' N, 17° 05' W	NE trades	Red-brown	26	Winds NNE

* For samples GH1 to GH6 the location given is the ship's position at the start and finish of each collection.

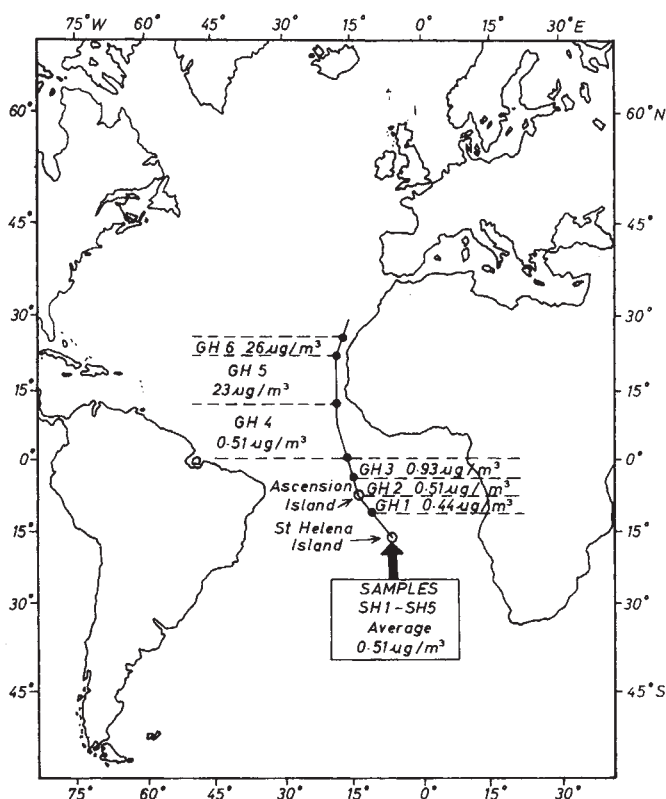


Fig. 1 Collections of atmospheric dusts in the south-east and north-east trade winds. The solid line indicates the partial track of the RMMV Good Hope Castle. The sample reference numbers are given together with the atmospheric dust loadings (calculated assuming a 50% mass collection efficiency).

result of an injection of material into the local atmosphere from the islands of St Helena and Ascension. Dusts GH5 and GH6 have a particle size distribution very similar to that of other dusts collected in the same area (Table 2). Sample GH4 has a larger proportion of small sized particles than either GH5 or GH6. The colour and mineralogy of GH4 indicate that a large proportion of the dust has the same origin as GH5 and GH6, that is, West African deserts, but it will have lost some larger sized material because it has been transported for a greater distance.

The mineralogy of some of the dusts is given in Table 3. The mineralogy of aeolian dusts has been widely reported in

the literature^{1,2,3,5} and only those features which highlight the differences between dusts of the NE and SE trades will be mentioned here. (1) It has been suggested¹ that dolomite probably from the Sahara Desert region characterizes NE trade wind dusts. Our data further indicate the usefulness of this mineral as an indicator of a Sahara Desert origin as it was not identified in any of the dusts from the SE trades; (2) the principal difference in the $< 2 \mu\text{m}$ clay mineralogies of the NE and SE trade wind dusts is that montmorillonite is far less abundant in the last of these. In South Atlantic deep-sea sediments montmorillonite has its highest concentration in mid-ocean areas where it constitutes between 30% and 50% of the total $< 2 \mu\text{m}$ clay minerals⁷. This has been interpreted in terms of either an aeolian input of montmorillonite or the *in situ* formation of this mineral from volcanic material of the Mid-Atlantic Ridge^{7,8}. Our results indicate a relatively low montmorillonite content for South Atlantic dusts and support the hypotheses of the *in situ* formation of this mineral from local volcanic debris.

Our data and those given in the literature allow a tentative comparison between the dust-carrying capacities of the NE and SE trades of the Atlantic Ocean. The NE trades have a large reservoir of loose surface deposits in the desert areas of West and North Africa. Such an origin is reflected in the reddish colour usually shown by these NE trade dusts. Both the Kalahari (an inland desert) and the Namib (a coastal desert), both in southern Africa, could act as a source of dust for the SE trade winds. It has been shown^{1,9} that dust originating in the Sahara Desert can be transported in relatively large quantities about 6,000 km westwards across the Atlantic. For example, dust-loadings at Bermuda in summer averaged about $6 \mu\text{g m}^{-3}$ of air. The influence which the Sahara reservoir exerts on the dust loadings of the NE trades is apparently much greater than the combined effects of the Kalahari and Namib deserts on the SE trades. In the atmosphere off St Helena, which lies about 2,500 km from the coast of South West Africa, the average dust loading is approximately $0.51 \mu\text{g m}^{-3}$ of air. This dust loading was obtained from collections made in the southern hemisphere winter; dust loadings will vary considerably from day to day and season to season depending on local meteorological conditions. From the haze-frequency distribution maps given by MacDonald¹⁰ it can, however, be seen that the 5% isopath extends further from the coast of Africa in the southern hemisphere winter than it does in summer, that is, the winter dust loadings which we have obtained are likely to be an average upper limit for the yearly South Atlantic dust concentrations in this area.

To assess fully the effect of the Kalahari and Namib deserts on dust loadings of the SE trades, collections must be made southwards of St Helena towards the Namib coast of South

Table 3 Mineralogy of Some of the Dusts

Dust	Clay mineralogy* ($< 2 \mu\text{m}$ fraction)					$> 2 \mu\text{m}$ fraction†							
	Montmorillonite	Illite	Chlorite	Kaolinite	Mica	Talc	Amphiboles	Chlorite	Kaolinite	Quartz	K-feldspar	Plagioclase	Dolomite
SH1	Only small amounts of these samples were collected, and the $< 2 \mu\text{m}$ fractions contained a high proportion of X-ray amorphous material					x	x	—	Trace	x x x	—	x x x	—
SH2						Trace	—	Trace	Trace	x x	—	x x x	—
SH3						x	x	x	?	x x	—	x x x	—
SH4						x	x	Trace	Trace	x	—	x x x	—
GH1	Trace	43	57		x	x x	Trace	—	—	x x	—	x x x	—
GH2	Trace	46	31	23	x	x	—	x	—	x x	—	x x x	—
GH3	2	25	11	62	x x	x	—	x	x	x x x	x x x	—	—
GH4	20	38	14	28	x x x	x	x	x x	x x	x x x	x x x	x x x	—
GH5	43	22	17	18	—	—	—	—	—	—	—	x x	—
GH6	10	41	17	32	x	—	Trace	x	x	x x x	x x x	x x	x x x

* The analyses are in terms of a 100% clay sample.

x, Present; x x, abundant; x x x, very abundant; —, not found.

West Africa. Our data does indicate, however, that in mid-ocean areas the deposition of eolian material from the SE trades probably has a much smaller effect on deep-sea sedimentation in the South Atlantic than it does from the NE trades of the North Atlantic.

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Recognition of Upper Miocene Neogene Zone 18, Experimental Mohole, Guadalupe Site

CORRELATIONS of marine strata using the convenient numerical zones of the Neogene^{1,2}, based on planktonic foraminiferans, represent an important advance in refining and developing greater precision in biostratigraphic correlations. This zonation is most effective in tropical and subtropical regions where the nominate planktonic taxa are best developed; progressive difficulty is encountered in applying it away from equatorial areas as these taxa disappear and are replaced by temperate and then by cold-water populations in polar areas. The experimental Mohole (Fig. 1), drilled near Guadalupe Island off Baja California, is significant as far as tropical-temperature planktonic foraminiferal populations are concerned because it is located in a subtropical area in which there is a commingling of tropical and temperate planktonic species³.

Although studies have already been made of the experimental Mohole foraminiferans³⁻⁵, the section was reanalysed in terms of Neogene Planktonic Zones (Fig. 2). The uppermost Miocene zone, Neogene Zone 18, is of special importance for three chief reasons which are relevant to the geologic history of the eastern Pacific. First, it is the uppermost zone of the Miocene and it is

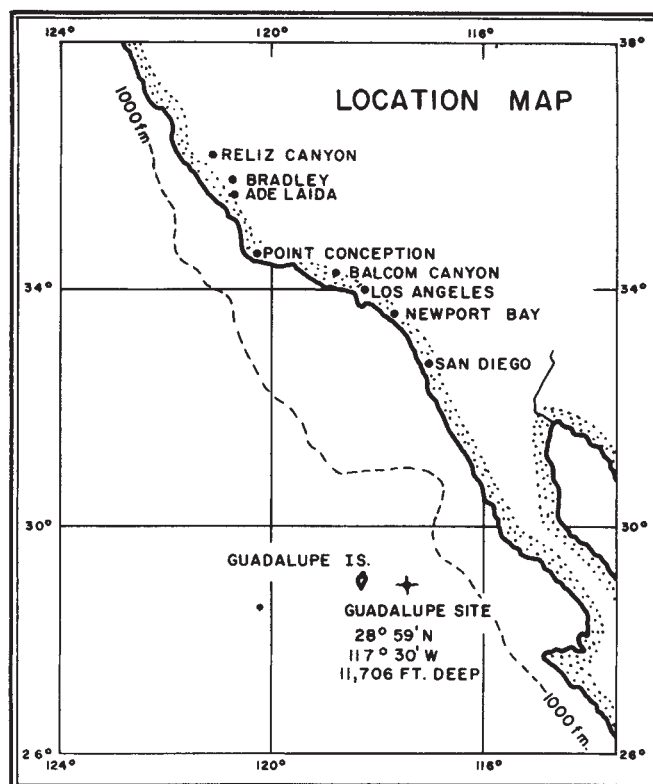


Fig. 1 Location of the experimental Mohole (Guadalupe site).

now possible to correlate Neogene Zone 18 of the standard numerical system for the tropics with the experimental Mohole section and in turn with the uppermost Miocene of California—the uppermost Mohnian and Delmontian Stages—using concurrent ranges of the tropical species *Globorotalia tumida plesiotumida* Banner and Blow (Fig. 3) and its descendant *Globorotalia tumida tumida* (Brady)⁶. Neogene Zone 18 can also be defined in the uppermost Messinian, the type upper Miocene of Italy^{7,8}; this is a critical aspect for interregional correlations. Second, Neogene Zone 18 has been correlated with the upper Gilbert and lower Gauss Magnetic Epochs⁹, the upper boundary being approximately at 3 m.y. or perhaps slightly more. This is in agreement with the 4.3 m.y. dating¹⁰ for the Delmontian of the Mohole (Fig. 2). Third, the opening of the Gulf of California about 4 m.y. ago¹¹ allowed late Miocene-early Pliocene planktonic biofacies to enter the Gulf and to penetrate to the head of the Gulf before the end of the Miocene; the development of the northern Gulf before the opening at the mouth, as suggested by Larson and others¹¹, is not required.

The marine deposition rates of many land-based sections are related to the definition of the upper limit of Neogene Zone 18, uppermost Miocene, with respect to southern California geology. The Ventura Basin¹², for example, has between 5,000 and 6,000 m of Pliocene-Pleistocene strata and the Los Angeles Basin¹³ has not more than 3,000 m of section in the Pliocene-Pleistocene. Correlation of Neogene Zone 18 with the uppermost Gilbert and lower Gauss Magnetic Epochs suggests that there is a deposition rate of between 1,000 and 2,000 m per million years¹⁴. These basins, which are actively subsiding and have nearby sediment sources, exhibit rates from two to more than four times greater than those suggested for the Plio-Pleistocene of New Zealand¹⁵. They are compatible with rates indicated for the modern Santa Barbara Basin¹⁶ and are many times greater than those computed for the Miocene of California.

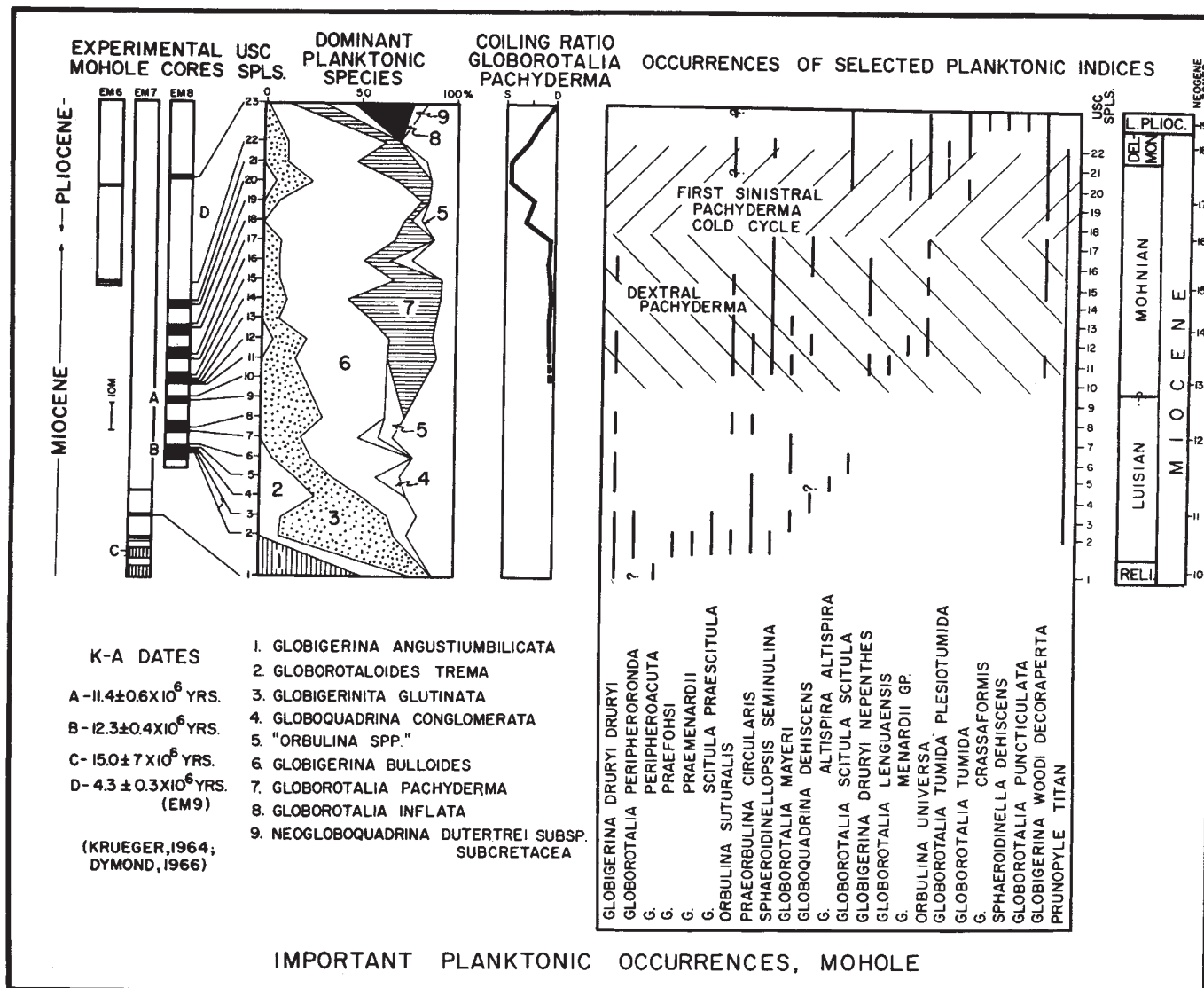


Fig. 2 Neogene planktonic foraminiferal zones (right side of figure) with important planktonic indices of the experimental Mohole showing coiling ratios of *Globorotalia* (*Turborotalia*) *pachyderma* (S, sinistral coiling; D, dextral coiling), the frequency distribution of common and abundant species, and the relative rare species as a bar graph. All are planktonic foraminiferans except *Prunopyle titan*, which is a radiolarian. K-A dates A, B, and D are those of Dymond¹⁰ and C is from Kreuger¹⁷. This figure is from Bandy⁸ as restudied and modified from that of Bandy and Ingle⁹. As noted in more complete analyses⁸, Neogene Zone 13 marks the development of *G. (T.) pachyderma* just above the *Discoaster kugleri* zone; Neogene Zone 14 is defined by the concurrence of *Globorotalia (T.) mayeri* and *Globigerina druryi nepenthes*; Neogene Zone 17 is the level of maximum cooling, indicated by the dominance of sinistral populations of *G. (T.) pachyderma*; and Neogene Zone 18 is defined by the concurrence of *G. tumida tumida* and *G. tumida plesiotumida*.

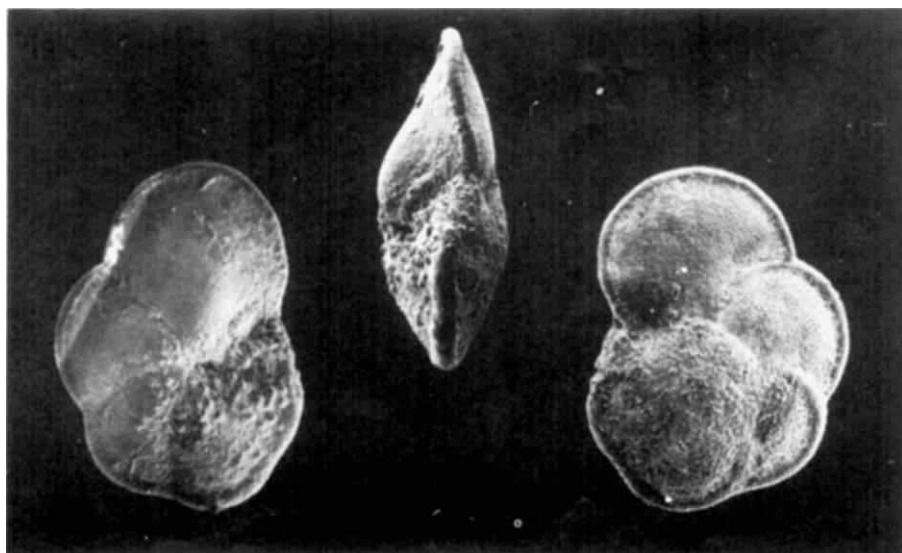


Fig. 3 *Globorotalia tumida* (Brady) sub-species *plesiotumida* Banner and Blow from USC sample 21, experimental Mohole section, Guadalupe site (Fig. 2). Specimen has a maximum diameter of 0.43 mm.

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BIOLOGICAL SCIENCES

Allosteric Preconditioning: Role of Allosteric Ligands in Promoting the Maturation of Enzymes

THE tertiary structure of an isolated polypeptide chain may differ from its most stable conformation as a component of an oligomer. The assembly of free subunits into an oligomeric structure may therefore involve a relatively unstable oligomeric intermediate state in which the conformation of the subunits corresponds to that of the free polypeptide chains. Such a metastable intermediate state may be separated from the stable oligomer by a substantial energy barrier, and the marked temperature dependence of subunit association reactions has indeed been attributed to the existence of a "conformational transition state"¹.

We wish to focus attention on the surmounting of this energy barrier, that is, the transformation of an immature precursor of the enzyme, devoid of activity, into a form capable of catalysis (in the case of apoenzymes, a form capable of combining with the prosthetic group to yield an active enzyme). This process, which can be termed preconditioning, often seems to be affected by the ligands that also regulate the activity of the mature enzyme. We believe that this process, allosteric preconditioning, represents another general mode of regulation by allosteric ligands. Five specific examples reveal fundamental differences between the manner in which allosteric ligands affect preconditioning and the way in which they modulate the activity of the mature enzyme.

(1) Threonine deaminase, the biosynthetic enzyme from *Salmonella typhimurium*, can be reconstituted from the apoenzyme and pyridoxal phosphate. The resulting holoenzyme is, however, inactive until subjected to a maturation process. The rate of this process, especially at pH 7.5 and above, is greatly enhanced by valine or isoleucine, which are positive

and negative effectors, respectively, of the active enzyme. On the other hand, the simultaneous presence of both effectors blocks the maturation process^{2,3}.

(2) Muscle phosphofructokinase can be inactivated by various means, including mild acidification and prolonged storage (refs. 4–6, and manuscript in preparation). The basis for the inactivation by mild acid is considered to be a dissociation into two subunits^{4,6}. Activity is restored slowly at pH values above neutrality, but is markedly accelerated by certain ligands. ATP and ADP (which serve both as substrates and allosteric modifiers of this enzyme) are effective in the restoration of activity, but less so in the presence of Mg²⁺. The positive effectors AMP and cyclic 3,5'-AMP also promote restoration. That it is the inhibitory site for ATP and not the substrate site that is involved in the preconditioning process is suggested by the interference of Mg²⁺ and by the fact that ITP, which is an excellent substrate but a poor inhibitor of this enzyme⁷, is much inferior to ATP in reactivating the enzyme (manuscript in preparation).

(3) The behaviour of glycogen synthetase can be modified by phosphorylation and dephosphorylation, but the enzyme is also susceptible to the kind of conditioning we are concerned with. Thus, it has been shown with the liver enzyme that there are two mechanisms for the conversion of the D-form (dependent on high concentrations of glucose-6-P) to the I-form (relatively independent of glucose-6-P). One mechanism, which has been termed "direct", presumably involves phosphatase action. The "indirect" conversion, which is relevant here, proceeds through an intermediate, inactive state (produced by incubation at moderate temperatures in the presence of EDTA at alkaline pH⁸). Maturation of this to a catalytically active enzyme resembling the I-form is rapidly promoted by Mg²⁺ and SO₃⁼, and by the allosteric effector of this enzyme, glucose-6-P^{8,9}.

A similar situation is observed in the case of pyruvate kinase. The enzyme from adipose tissue, which is dependent on FDP, can be rendered independent of that modifier by preconditioning in the presence of FDP. Interestingly, ATP, an inhibitor of the enzyme, interferes with the FDP-mediated preconditioning¹⁰.

(4) Pyruvate carboxylase seems to provide an example of the preconditioning of an apoenzyme. A specific synthetase catalyses the transfer of biotin to apo-pyruvate carboxylase isolated from *Bacillus stearothermophilus*. This reaction is strongly dependent on acetyl CoA, which is also a positive effector of the holoenzyme. On the other hand, L-aspartate antagonizes the effect of acetyl CoA both in the synthetase reaction and in the carboxylation *per se*^{11,12}.

(5) Tryptophan pyrrolase provides a classical example of substrate-mediated enzyme maturation¹³. The rate of production of active holoenzyme from apoenzyme is enhanced by tryptophan about ten-fold¹⁴, but the concentration of tryptophan required for half-maximal rate of apoenzyme combination with haemin is considerably below the *K_m* for tryptophan of the holoenzyme; moreover, α-methyltryptophan, neither a substrate nor an inhibitor of the holoenzyme, can effectively promote maturation¹⁴. This suggests that preconditioning of tryptophan pyrrolase involves the binding of tryptophan to an "allosteric" site rather than to the active site of the enzyme.

These examples reveal an important general feature of allosteric preconditioning: although allosteric ligands may promote the process, it can also occur in their absence, albeit much more slowly. Moreover, the continued presence of the maturation-inducing ligands is not required for the maintenance of the mature state, that is, maturation is essentially irreversible and thus the mature state must represent the thermodynamically more stable form of the enzyme. Allosteric ligands must therefore promote preconditioning not by stabilizing the mature enzyme but by enhancing the rate of conversion of the immature to the mature state (Fig. 1). This role of allosteric ligands differs fundamentally from their role in allosteric activation or inhibition of enzyme activity, which

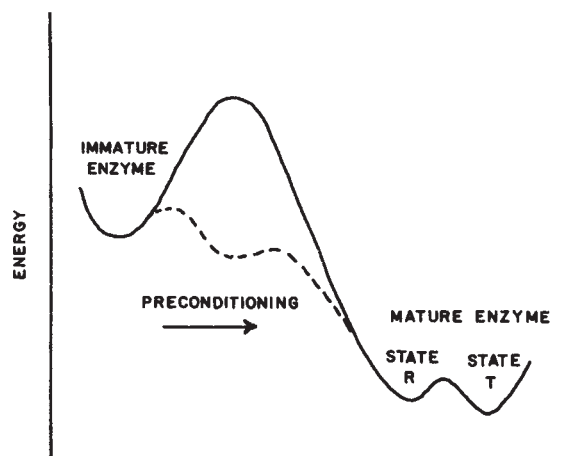


Fig. 1 Preconditioning of an enzyme in the absence (—) and in the presence (---) of a maturation-promoting ligand.

is mediated by the preferential stabilization of either an active or inactive form of the enzyme (that is, states R and T in Fig. 1)¹. This difference is also illustrated by the fact that preconditioning, unlike allosteric inhibition or allosteric activation, can be promoted by either positive or negative effectors. Thus, either the positive effector valine or the negative effector isoleucine induces maturation of threonine deaminase, and phosphofructokinase is reactivated by adenine nucleotides including both positive and negative modifiers. Yet, in spite of the different mode of action of effectors in allosteric regulation of enzyme activity and in promoting enzyme maturation, it seems likely that in both processes the effectors bind to the same stereospecific site(s) on the enzyme. Direct evidence for this hypothesis must come from the study of the preconditioning of enzymes in which allosteric sites are altered by chemical modification or mutation. The observation that ATP promotes the conditioning of phosphofructokinase more effectively than ITP, which is in accord with the relative affinity of these nucleotides for the inhibitor site, supports such an idea.

Since allosteric preconditioning and allosteric regulation seem to involve the same site on the enzyme, it is important to distinguish allosteric preconditioning from the frequently observed stabilization of enzymes by allosteric ligands. The latter can be attributed to the fact that the catalytic centre of an enzyme may be more stable in one of the allosteric states of the enzyme (usually the inactive state), and thus any ligand that binds preferentially to this state will enhance the stability of the enzyme. The observation that either valine or isoleucine can promote the maturation of threonine deaminase, in spite of the fact that the positive effector valine (unlike isoleucine) reduces the stability of the enzyme¹⁵, lends validity to this distinction.

If preconditioning or maturation were indeed obligatory in the biosynthesis of an active enzyme, as seems to be the case at least with threonine deaminase, pyruvate carboxylase and tryptophan pyrrolase, one might conclude that the primary function of allosteric sites is the binding of maturation-promoting ligands. Once such a site has evolved on a protein, the mature form of which could exist in several conformational states that differ in catalytic activity, it would be likely that the affinity of this site for its ligand would differ for each state, a situation that would subject the catalytic activity of the enzyme to regulation by the maturation ligand. In other words, allosteric regulation would be the frequent outcome of ligand-promoted preconditioning. Nevertheless, enzymes should exist where this evolution of preconditioning sites into regulatory sites has not yet occurred. Such an example may well be provided by liver tryptophan pyrrolase, where α -methyl-

tryptophan promotes maturation by binding to a separate site but yet has no influence on the activity of the holoenzyme. On the other hand, in the enzyme from *Pseudomonas acidovorans*, the preconditioning site seems to have evolved into a regulatory site, for here α -methyltryptophan not only induces maturation but also acts as a positive allosteric effector of the holoenzyme¹⁶.

The presence of maturation sites that have not yet evolved into regulatory sites may also account for occasionally observed enzyme-ligand interactions that are difficult to explain in physiological terms. For example, FDP specifically increases the thermolability of *Escherichia coli* β -galactosidase¹⁷ but has no effect on the enzyme's activity. (Yet FDP is known to be an allosteric modifier of another catabolic enzyme in *E. coli*—glycerokinase¹⁸.) Another effect which is difficult to rationalize physiologically is that of nonpolar L-amino-acids on aspartokinase. In *B. polymyxa*, nonpolar L-amino-acids act as weak activators¹⁹, in *E. coli* as weak inhibitors²⁰, and in *B. subtilis* some nonpolar amino-acids activate while others inhibit aspartokinase (manuscript in preparation). These ligands may play a role in enzyme maturation rather than in the regulation of activity, and it should be of interest to determine whether the reconstitution of the enzymes from their polypeptide chains is not enhanced by these specific agents.

Interaction of enzymes with allosteric ligands is not the only way to effect preconditioning. As mentioned, variation of pH or the presence of various ions can also accelerate enzyme maturation, and substrates or coenzymes can also promote this process, as observed with several dehydrogenases²¹⁻²³. We have focused our discussion on the role of allosteric effectors because the cited examples encourage the hypothesis that the preconditioning process may often be the antecedent of allosteric regulation. Moreover, the concepts proposed would provide a unified view of preconditioning phenomena and stimulate the search for such a process among allosteric enzymes and especially among those proteins which exhibit hitherto unexplained interactions with specific small molecules.

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Chromosomal Localization of the Heterochromatic Region 16qh(.76) linked to α -Haptoglobin in Man

THE structural gene locus for the alpha chain of haptoglobin (α -Hp) in man seems¹ to be on the long arm (q) of chromosome 16 linked² to a fragile heterochromatically staining region (h). We here report measurements localizing this region, which we designate 16qh(.76).

16qh(.76) was found to be segregating in a large family². From thirty-one of the family members heterozygous for 16qh, 1,709 lymphocyte metaphases were analysed microscopically and 801 metaphases photographed. Both chromosomes No. 16 were relatively straight and free of overlap by other chromosomes in 193 metaphases. Eighty of these metaphases showed 16qh clearly and were therefore selected for measurement.

Using photographic prints with a final enlargement of $\times 3,840$, we measured the No. 16 chromosomes with a specially designed, manually operated wheel on which a millimetre scale was directly engraved (unpublished results of H. W., W. J. K., and F. H.). Both chromatids were measured and the results averaged. We measured the length of the short arm (p) from each chromosome No. 16, q from the normal homologous 16 ($q^{\text{homol.}}$), and the euchromatic parts of q proximal ($q^{\text{prox.}}$) and distal ($q^{\text{dist.}}$) to 16qh.

We compared the length of $q^{\text{prox.}}$ to $q^{\text{homol.}}$ in each cell and found the mean length of $q^{\text{prox.}}$ to be 0.759 of $q^{\text{homol.}}$ (s.e. = 0.011). Thus, the fragile heterochromatic site is approximately 76% of the distance along the long arm of No. 16. Since other heterochromatic regions may be located on 16q, we have designated this site 16qh(.76).

Table 1 One Way Analysis of Variance of the Length of the Distal Segment of the 16qh Chromosome

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio
Between person	31.0	0.6631	0.0213	
Within person	48.0	0.7215	0.0150	
Total	79.0	1.3846		1.422

We then tested whether 16qh(.76) detectably affected the length of euchromatin on that chromosome, using a paired observation *t* test. The mean length of p from the involved No. 16 was not significantly different from the mean length of p from the homologous ($t=1.68$, $df=79$, $P>0.05$). The sum of the means of $q^{\text{prox.}}$ + $q^{\text{dist.}}$ was, however, 2.6% greater than the mean of q from the homologous ($t=2.17$, $df=79$, $0.05>P>0.02$).

Finally, we tested for heterogeneity between persons in the length of 16q^{dist.} and found none. The F ratio (between person/within person variance) was 1.42 (Table 1), which is not significant.

The foregoing cytological data should not be interpreted to mean that the structural gene for α -Hp is necessarily located 76% of the distance from centromere. α -Hp could be cytologically at some distance from h(.76) but seem to be closely linked, if the heterochromatic region tended locally to suppress crossing-over. The data also cannot reveal if α -Hp is between the centromere and 16qh(.76) or distal to 16qh(.76).

Concurrent segregation analysis of chromosome markers (for example, 16qh(.76)) and gene markers (for example, α -Hp) permits the testing of map assignments deduced from studies with chromosome rearrangements¹ and the localization of specific genes to specific chromosome regions, as in the present study. The efficiency of chromosome marker work will predictably increase as chromosome variants are recognized more frequently. In this family we have recently observed the segregation of three other potentially useful variants (3 inv, 17qh and Ginv), as will be reported in more detail.

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Interpretation of Linkage in Somatic Cell Hybrids

Kao and Puck¹ have measured the linkage between human genes by studying their association in cell hybrids with Chinese hamster cells which carried mutations homologous to the human genes. Such studies are made possible by the extensive loss of human chromosomes from such hybrids. If, however, one of the human genes is retained owing to the operation of specific selection, the presence or absence of an unselected gene gives a measure of linkage between them.

In one hybrid studied by Kao and Puck the human genes were inositol independence (*inos*⁺) and proline independence (*pro*⁺). Among forty-two clones selected for *inos*⁺, only seven (one sixth) were also *pro*⁺. In another hybrid the human genes were glycine independence (*gly*⁺) and *pro*⁺. Among fifteen clones selected for *gly*⁺ only five (one third) were also *pro*⁺. Both these experiments were interpreted as evidence of non-linkage between the pair of marker genes. I submit that such associations, though well below the 50% expected between unlinked genes in sexual hybrids, are nevertheless greater than would be expected from random association of the marked chromosomes in cell hybrids. The first problem is to determine what would be the extent of random association between two unlinked genes (that is, genes on separate chromosomes). This would depend on the number of human chromosomes still retained in the hybrid. In the first hybrid, because of the method of selection one chromosome must carry *inos*⁺. If a second chromosome is present the probability that it will not carry *pro*⁺ is 43/45. The chance that a third and fourth chromosome will also be free of *pro*⁺ will be 42/44 and 41/43 respectively. In general terms, if a hybrid clone has *n* human chromosomes of which one carries *inos*⁺, the chance of *pro*⁺

being also present will be $1 - \frac{43! \dots (45-n)}{45! \dots (47-n)}$. When $n=2$ the association of *inos*⁺ and *pro*⁺ will be 0.044 and increases

approximately in proportion to $(n-1)$. The observed association of 0.167 would require an average of 5 human chromosomes in all clones. The association in the second hybrid of 0.33 between *inos*⁺ and *gly*⁺ would require the presence of ten human chromosomes, but Kao and Puck show an idiogram with seventeen hamster chromosomes and two "human" chromosomes (judged to be 2 and 6 or X under the Denver classification) and state that other clones had "similar karyotypes", and that all stabilized clones had nineteen, or occasionally twenty, chromosomes. The original Chinese hamster cell line had twenty chromosomes, so it seems that two human chromosomes have been substituted for three hamster ones. One is forced to conclude that the human chromosomes retained in the hybrids are much fewer than five, and the association found between the genes cannot be attributed to chance association of two chromosomes. Although there is only one kind of random association of chromosomes, there is a large number of non-random associations which might be considered. The simplest and most probable form of non-random selection arises from the fact that we are sampling from a diploid set with two representatives of each kind. It would seem likely that if a sample of chromosomes contains the haploid number (twenty-three) or less, it will not contain more than one of each homologue. This would produce only a slight modification of the fully random formula given above. The association of two markers among n chromosomes would become $1 - \frac{21! \dots (23-n)}{22! \dots (24-n)}$. For an association of 0.167, n is still 5, and for an association of 0.33 it is 9. Such non-randomness is not, therefore, capable of explaining the associations found.

Another kind of non-randomness would arise if, though the chromosomes were lost at random, certain combinations were more viable than others. In cases where one chromosome was selected, the probability of any particular second chromosome being present would vary from more than random (0.044) to less than random according to the viability of the combinations. In the hybrids considered here, the *pro*⁺ chromosome would seem to have a greater than random chance (four-fold and eight-fold respectively) of being included with the selected chromosomes *inos*⁺ and *gly*⁺.

Another non-random model assumes that human chromosomes are of two kinds, compatible and incompatible with the Chinese hamster genome. The hybrid karyotype suggests that compatible human chromosomes might be those which can be substituted for hamster chromosomes. If all the compatible chromosomes had equal chances of inclusion in the hybrids and their number was x , the chromosomes carrying *inos*⁺, *pro*⁺ and *gly*⁺ must be among that number. The chance of any one compatible chromosome carrying *pro*⁺ will therefore be $2/x$. The first chromosome has been determined by the method of selection. The chance that the second one will carry *pro*⁺ will be $2/(x-2)$. The data give us two estimates of x : 14 when *inos*⁺ is selected and 8 when *gly*⁺ is selected, with a best estimate of 11. In other words, out of forty-six human chromosomes only eleven are compatible with the Chinese hamster genome. The concept of incompatible human chromosomes opens up further possibilities. If the gene being selected for in the hybrid were on an incompatible chromosome, it could be retained, but only by a structural change; by being translocated onto a host chromosome, onto a compatible chromosome of the other parent, or by deletion of the "incompatible segment". It would then become very difficult to identify the chromosome carrying the human gene and even more difficult to identify the chromosome from which it was derived. Pursuing the model further, the association found between genes could be due to their presence on the same human chromosome, the degree of association being determined by the classical map distance between them; that is, by the chance that the break which enables the selected gene to be retained in the hybrid will also include the associated gene.

The karyotype presented as typical by Kao and Puck was interpreted by them as having three hamster telocentrics replaced by two human metacentrics. Another possibility is that the two new chromosomes represent rearrangements between three hamster telocentrics and one arm of a human chromosome. Such spontaneous rearrangement may seem an unlikely explanation but many spontaneous rearrangements accompanied the origin of the permanent Chinese hamster cell line, eight out of twenty chromosomes of which do not resemble any chromosomes in the primary explant. Moreover, the extreme selection pressure operating in favour of human wild type genes would fix any rare events which increased their chance of survival. This is the probable explanation of the high degree of dissociation in man-mouse hybrids between two human genes known to be sex-linked (HGPRT and G6PD) when only one of them is subject to positive selection². If the association of *pro*⁺ with *inos*⁺ and with *gly*⁺ is due to location on the same human chromosome, the strength of the association indicates the map distances and the gene order, which would be *pro gly inos* or *gly pro . . inos* (*inos* being twice as far as *gly* from *pro*).

This model fits evidence from an entirely different source. It has been argued³ that the isolation of recessive mutants in Chinese hamster near-diploid cell lines is due to the existence of chromosome segments in the monosomic condition, so that there are no dominant wild type alleles to mask the mutations. Such mutations would be confined to few segments or even to a single chromosome segment. The location of the mutant genes *pro*⁻ *inos*⁻ and *gly*⁻ on one segment of hamster chromosome would then be paralleled by the location of the genes *pro*⁺ *inos*⁺ and *gly*⁺ on one segment of human chromosome.

To conclude, the association found by Kao and Puck between *inos*⁺ and *pro*⁺ and between *gly*⁺ and *pro*⁺ in Chinese hamster/human hybrids is greater than would be expected from the random association of two chromosomes. Three possible causes of such an association of the human genes have been considered.

If the associated pairs of genes are on different human chromosomes, these chromosomes must be associated, (a) because of some genetic interdependence, such that the presence of one increases the probability that the other will also be present; (b) because of some specific compatibility that both share with the hamster genome (for example, ability to substitute for hamster chromosomes); (c) both genes may be located on the same human chromosome, but this chromosome has a high incidence of rearrangement in hybrids. Theoretically, these explanations seem to be equally valid. They require to be tested by further genetic studies in cell hybrids combined with intensive karyotyping.

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Activation of a Central Noradrenergic Projection to Cerebellum

RECENT histochemical studies have demonstrated abundant nerve fibres containing noradrenaline in rat cerebellar cortex^{1,2} terminating predominantly on Purkinje cells². An inhibitory function for these fibres is suggested by the uniform inhibition

of the spontaneous discharge³ and hyperpolarization of Purkinje cells (P cells) produced by microelectrophoretic application of noradrenaline (NA)⁴. Furthermore, the firing rate of Purkinje cells is generally increased after destruction by 6-hydroxydopamine of fibres containing NA⁵. Further characterization of the natural endogenous transmitter and its effect on P cells requires experimental activation of the proposed adrenergic pathway, and identification of the origin of the NA projection to the cerebellum thus assumes critical importance. It has been suggested that the nerves containing catecholamine in rat cerebellar cortex arise largely from discrete bilateral nuclei of fluorescent cells in the dorsal medullary brain stem (the locus coeruleus, LC)^{6,7}. We have reported that electrical stimulation in the area of the LC inhibits P cells⁸. We now report that this inhibitory response is evoked selectively from the NA-containing cells of the LC, that the inhibitory effects are widespread and that the response closely resembles the electrophysiological effects produced by exogenous NA.

Male albino rats (130–230 g) were anaesthetized with halothane. The cerebellar vermis and one hemisphere were exposed³. A fine (0.4 mm) concentric bipolar stimulating electrode was inserted through the hemisphere at an angle of 29° from the vertical into cerebellar and brain stem loci after empirical stereotaxic localization of the LC. The location of the electrode was verified histologically after each experiment. Purkinje cells, identified by their characteristic climbing fibre bursts⁹, were recorded in the cerebellar vermis with extracellular micropipettes (2 M NaCl, 1–8 MΩ) or intracellular micropipettes (3 M KCl, 10–40 MΩ). Responses to brain stimulation (0.1 ms pulse duration) were recorded on magnetic tape. Mean firing rate (spikes s⁻¹) was displayed with a pen recorder³. Post-stimulus time histograms (PSTHs) were generated by a Digital PDP-12 computer. Mean arterial pressure was monitored during LC stimulation and showed no change.

Purkinje cells, but not cortical interneurons, showed a remarkably uniform inhibitory response to stimulation of LC with trains of pulses: ninety-four of 102 cells (twenty animals) recorded extracellularly displayed depression of spontaneous discharge rate (Fig. 1*A, B*). This summated response was greatest at relatively low stimulus frequencies (3–50 s⁻¹) and was markedly diminished at faster rates. Complete cessation of discharge outlasting the stimulation period by 4–65 s (mean, 21 s) could be obtained with 20–100 pulses at 10 s⁻¹. At this frequency, threshold currents ranged from 0.03 to 1.2 mA (mean, 0.35 mA).

Although the response to a single LC stimulus often escaped detection on direct visual inspection, construction of PSTHs reproducibly revealed reduced probability of spike discharge over prolonged intervals of 60 to 470 ms (mean, 293 ms) with long latent periods of 50–290 ms (mean, 148 ms). During these late inhibitions, cell discharge was often only incompletely suppressed; however, a single short burst of two to five pulses at high frequencies (20–75 s⁻¹) usually resulted in a complete pause in firing (Fig. 1*C*).

Stimulation of LC often produced short term (5–10 ms) activations of P cells (preceding the late inhibitory period), consisting either of single spikes, climbing fibre bursts, or, more frequently, simple or complex field potentials without obvious spikes. These rapid responses had latent periods ranging from 1.5–11 ms, and were often immediately followed by short inhibitory periods of 10–60 ms (mean, 18 ms). These early events may result from current spread to other brain stem areas (for example, brachium conjunctivum), because (i) many P cells exhibiting late inhibition showed no such responses, and (ii) dissociation between the long inhibitory responses and the activations and/or fields could often be produced by fine adjustment of the stimulating current or by specific drug administration (B. J. H., G. R. S., F. E. B., and A. P. O., in preparation). However, a direct connexion (via collaterals, for example) from LC to some cells of origin of mossy or climbing fibres cannot be ruled out at present.

Intracellular recording of some P cells during stimulation of

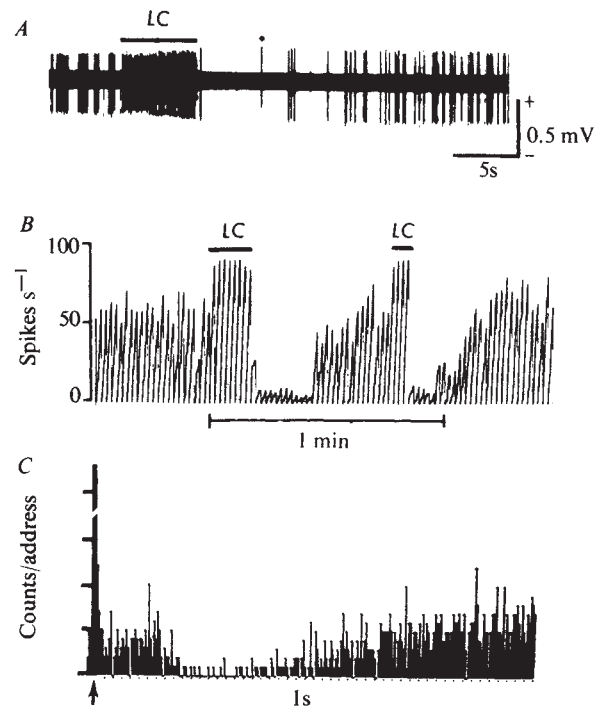


Fig. 1 Extracellular responses of P cells to stimulation of locus coeruleus (LC). *A*, Oscillograph record of action potentials; response to fifty-seven shocks (0.25 mA) delivered to LC at 10 s⁻¹ (solid bar above action potentials). A climbing fibre response to a single LC test stimulus is indicated by the dot above the record. *B*, Polygraph record showing integrated discharge frequency of another Purkinje cell. Firing rate drops to zero with multiple shocks (100 and 50 pulses, respectively; 10 s⁻¹, 0.32 mA) to LC (solid bars above record). Apparent excitation seen during stimulation results from addition of the multiphasic stimulus artefact. *C*, Post-stimulus time histogram of the response of another P cell to single short bursts of shocks (0.28 mA, 3 pulses at 75 s⁻¹) delivered to the LC every 2.1 s (sixty-four sweeps). Arrow shows stimulus artefacts. Each marker on ordinate = 5 counts/address. Address or bin size = 4 ms.

LC with single shocks revealed late hyperpolarizations (not directly related to climbing fibre responses) which were usually weak or nearly lost in baseline noise (Fig. 2*A*, broad arrow). Signal averaging techniques better delineate these small late hyperpolarizing responses (0.5–2 mV), which are comparable in latency and duration to the late prolonged periods of suppression seen in post-stimulus histograms (Fig. 1*C*).

With trains of pulses, large hyperpolarizations extending well beyond the stimulation period and averaging 14 mV (range, 2–39 mV) were recorded (Fig. 2*B*). An index of membrane resistance was obtained by measuring the size of climbing fibre spikes (see refs. 4 and 10)—or in obviously injured cells excitatory postsynaptic potentials (EPSPs), and by measuring the potential deflexions produced by hyperpolarizing currents (0.5–1 nA, 40 ms duration) passed through the recording micropipette in conjunction with a Wheatstone bridge circuit¹¹. In all cases, input resistance, as measured by these two parameters, either increased (ten cells) or did not change (two cells; Fig. 2*B*) during the LC evoked hyperpolarizations. Hence, this hyperpolarization does not seem to result from activation of a conventional inhibitory pathway (for example, parallel fibre activation of basket cells), for a decrease in resistance would then be expected⁹. In this respect, LC stimulation exactly mimics the action of exogenously applied NA and cyclic AMP, which also produce hyperpolarizations without a decrease in membrane resistance⁴. But other mechanisms such as presynaptic inhibition, or disfacilitation, although less likely to evoke such

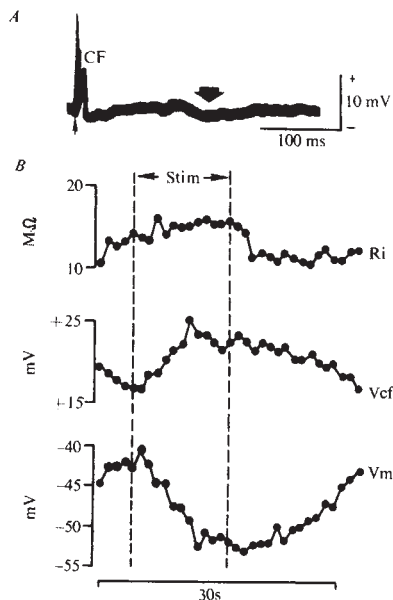


Fig. 2 Responses of P cells to stimulation of LC: intracellular recording. *A*, Oscillograph record of response to a single shock (small arrow; 0.7 mA). This supramaximal stimulus drives a climbing fibre evoked EPSP (CF) followed immediately by an early after hyperpolarization and finally a late, more prolonged hyperpolarization (broad arrow). *B*, Graph of intracellular response to stimulation of LC (Stim) with multiple shocks (0.45 mA, 120 pulses at 10^{-1}). Records were analysed at 1 s intervals by a PDP-12 computer, and the results for input resistance (Ri), size of climbing fibre responses (Vcf) and membrane potential (Vm) plotted against time. Although the input resistance did not change appreciably during this test, in the two other tests recorded in this cell, resistance increased by 164 and 295%.

large and widespread responses, might also produce the observed effects.

The anatomical discreteness of brain stem loci evoking this characteristic inhibitory response is underscored by the finding that the incidence of depression of P cell discharge falls abruptly when the stimulating electrode is placed slightly outside of LC (Table 1). In many cases, individual cells which responded when the electrode was in the LC failed to do so when the stimulation electrode was displaced from the LC by as little as 0.5 mm; recovery of response ensued with repositioning in the LC. Furthermore, when catecholamine-containing pathways were selectively and chronically destroyed by intracisternally-injected 6-hydroxydopamine^{2,12}, only five of sixty cells were inhibited (Table 1) when the LC was stimulated directly (seven animals) (B. J. H., G. R. S., F. E. B., and A. P. O., in preparation).

It might be argued that the late inhibitory responses to single shock stimulation of LC allow for conduction of impulses through many possible synapses, rather than the proposed monosynaptic noradrenergic pathway. A large portion of the

long latent period, however, may be due to a long synaptic delay, such as those of 30–100 ms described for the slow inhibitory postsynaptic potentials (IPSPs) of sympathetic ganglia^{13,14}. The slow IPSP, like the proposed LC-Purkinje cell inhibition, is conceived as an adrenergic response¹⁵. Moreover, the catecholamine-containing fibres to cerebellum are small C-fibres², which should conduct impulses very slowly. Conduction velocities for small peripheral C-fibres may range from 0.15 to 0.5 m s⁻¹ (ref. 16). Finally, the neuroanatomical data suggest a direct LC projection to P cells^{3,6-8}.

Furthermore, the LC evoked inhibition of P cells shares many properties of peripheral autonomic systems, such as the relative efficacy of trains of pulses over single shocks^{13-15,17}, a diminished responsiveness at high stimulus frequencies¹⁷, and hyperpolarizing responses without decrease in resistance¹⁵.

In view of early reports¹⁸ emphasizing the sparseness of catecholamine-containing fibres in the cerebellum, perhaps the most surprising finding of this study is that nearly all Purkinje cells recorded in the vermis exhibited the inhibitory response to LC stimulation. Almost all the cells in the LC of the rat contain noradrenaline¹⁹ and noradrenaline always inhibits Purkinje cells when applied exogenously³, so the proposed adrenergic projection from LC to cerebellar cortex may assume a greater functional significance than previously supposed. Pharmacological evidence supporting the noradrenergic nature of this projection will be reported separately.

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Table 1 Effect of Placement of Stimulus Electrode on Multiple Shock-evoked Pauses

Electrode location	Purkinje cells studied (n)	% Inhibited	Mean threshold current (mA ± s.e.)
Exactly within LC	102	92	0.35 (± 0.037)
0.3 mm outside LC	21	62	0.65 (± 0.082)
0.5–0.75 mm outside LC	15	33	—
> 0.75 mm outside LC	55	29	—
6-OHDA, in LC	60	8	—

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Regeneration of Porphyropsin *in vivo*

SCOTOPIC vision is mediated by the rod cells of the retina which contain a photosensitive pigment that (according to species) can be rhodopsin, porphyropsin, or a mixture of both. The pigment content may be diminished by exposure to light, but there is no permanent loss because the light-decomposed molecules are restored by regeneration. Although the time course and some details of the mechanism of rhodopsin regeneration *in vivo* are well known¹, we have little and contradictory information about the regeneration of porphyropsin. According to Kühne and Sewall² and Abelsdorff³, the porphyropsin-like pigment in the retina of the living bream (*Abramis brama*)^{4,5} is regenerated within 30–40 min. Garten⁶, however, who worked with the same fish, found regeneration to be complete after not less than 4 h. I have found a similar figure for regeneration of porphyropsin in the retina of crucian carp (*Carassius carassius*).

Crucian carp were used because their porphyropsin is not paired with rhodopsin⁷. In each experiment, four or eight animals of equal size (12–13 cm long) were light-adapted for 1 h in a transparent plastic tank (20 × 20 × 30 cm) illuminated by five reflector flood-lamps (light output per lamp, 1,950 lumens, colour temperature about 2,730 K). This light bleached about 70% of the pigment. The temperature during light adaptation was 16–17° C and 15.0 ± 0.3° C during subsequent dark adaptation. The dark periods lasted from 30 min to 5 h. To estimate the full pigment content of the fish eyes, some animals were dark-adapted overnight. Several precautions were taken to prevent regeneration during dissection and washing. After the fishes had been killed, the eyes were removed as quickly as possible and put on a block of solid carbon dioxide—a procedure which took about 4 min for six eyes. Subsequently, the retinas were dissected out into a Petri dish filled with an ice-cold solution (pH 4.5) consisting of a 1:1 mixture of McIlvaine buffer (pH 4.6) and 0.2 M hydroxylamine solution. The same cooled solution (2° C) was used to wash the retinas and the receptors. The 0.1 M hydroxylamine buffer solution served also as the solvent for the digitonin that was used as an extractant in a concentration of 3%. It was shown in some preliminary experiments that the relatively high concentration of hydroxylamine had no influence on the yield of pigment, nor did it diminish the stability of the extracts. Double extractions were made in all experiments, the first extracts contributing some 75% and the second ones approximately 20% to the total pigment yield.

The unbleached extracts exhibit two well defined absorption peaks at about 520 nm and between 355 and 360 nm (Fig. 1). The former is the easily identifiable porphyropsin peak, the latter is presumably caused by 3-dehydroretinol, the all-trans isomer of which has a $\lambda_{\max}$ of 350 nm. The second absorbance maximum shifts to shorter wavelengths when the extracts are prepared from retinas which have been left in darkness for comparatively short periods. In these conditions the ultraviolet peak was found near 352 or 353 nm and its height was somewhat greater. Although porphyropsin and presumably impurities contribute to the ultraviolet peak, its height may be taken as roughly proportional to the amount of 3-dehydroretinol present in the extracts (line B in Fig. 1). The absorbance change on bleaching at 520 nm (line A in Fig. 1), on the other hand, indicates the amount of porphyropsin present. The sum of A plus B was fairly constant in all the extracts ($(A+B) \pm \text{s.d.} = 1.2015 \pm 0.1964$; $N=13$) with the exception of those taken from fully light-adapted eyes. These were presumably contaminated with material extracted from the pigment epithelium, for it was difficult to obtain clean retinas in these experiments. The ratio of A to B increased with the duration of dark adaptation. A/B was as low as 0.05 in light-adapted retinas, it has the value 0.16 after 3 h of dark adaptation and approaches a figure of 0.30 in fully dark-adapted retinas (Fig. 1). If it is assumed that the material represented by the data was extracted chiefly from the receptors themselves,

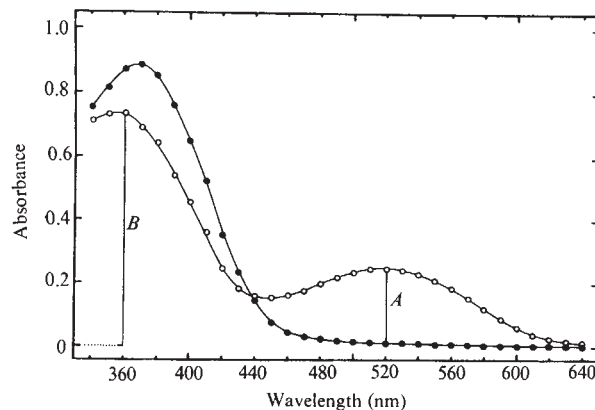


Fig. 1 Absorbance spectrum of a retinal extract from the dark-adapted crucian carp *Carassius carassius*. The data refer to a first extract from six retinas. The change from $\circ$ (unbleached) to $\bullet$ (bleached) is due to light exposure. The lengths of lines A and B are taken as respective measures of porphyropsin and 3-dehydroretinol.

then the values for A and B permit the following conclusions to be made: (1) the retina of *Carassius carassius* stores some 3-dehydroretinol; (2) 3-dehydroretinol released from bleached porphyropsin and reduced to the alcohol is added to the store; (3) during regeneration, the prosthetic group is supplied by this same store because the ratio A/B increases by a factor of six or so while the sum $A+B$ remains constant.

Difference spectra derived from curves, like those in Fig. 1, peak at 522 nm (positive branch) and 385–388 nm (negative branch) indicating that dehydroretinal oxime is the principal product of bleaching (compare ref. 8).

The time course of regeneration is shown in Fig. 2. The points represent the amount of photosensitive material ($\lambda_{\max} = 520$ nm) extracted from the retinas after various times in the dark. Although the data show appreciable scatter, the general trend is as follows: after a lag of approximately 1 h, regeneration begins slowly during the second hour. This is followed by 2 h of a fairly constant rate of increase of pigment until eventually regeneration approaches virtual completion during the fifth hour of darkness.

The mechanism that causes the long initial delay of regeneration is unknown. Regeneration of rhodopsin in the living frog is similarly delayed, but for not more than 15–20 min.

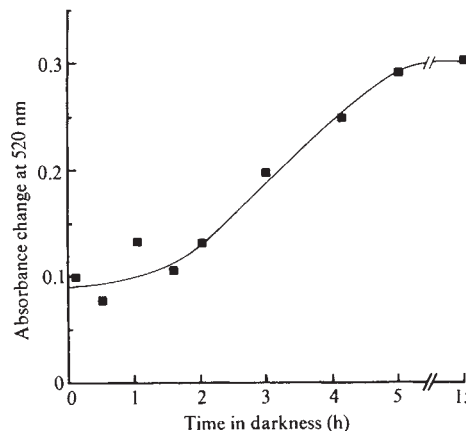


Fig. 2 Time course of porphyropsin regeneration in the retina of the living crucian carp. The ordinate refers to the amount of photosensitive material in extracts from six retinas, though two thirds of the data shown are based on double measurements with 2 × 6 retinas. Total number of experiments: fifteen.

This period, though shorter, is not of a different order of magnitude, however. Mammals, on the other hand, never show this lag¹. Their regeneration curve begins with the rectilinear part which the fish retinas exhibit during the third and fourth hour of darkness when the pigment is re-synthesized at the rate of 14% of the bleached porphyropsin in 30 min. This figure means that the regeneration of porphyropsin *in vivo* is slow⁶ and comparable with the regeneration of rhodopsin in cold blooded animals⁹. Three reactions are probably essential for the regeneration of the visual pigment: the formation of 3-dehydroretinal from the corresponding alcohol by oxidation, an isomerization of the retinal and the coupling of the prosthetic group with the protein. The first two reactions would involve two different enzymes—an alcohol dehydrogenase and an isomerase respectively. The course of consecutive enzymatic reactions is known to be complicated¹⁰, and a curve like that in Fig. 2 could well result if the two enzymes were present in different concentrations and their respective Michaelis constants were also different.

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L-Asparaginase and Complement

L-ASPARAGINASE, widely regarded as a potential oncolytic agent¹⁻⁶, is known to depress both cellular and humoral immunity. Our investigation of the effect of L-asparaginase on the complement system, described in what follows, reveals a more complicated interaction—the enzyme inhibits the complement in whole serum *in vitro* but is a potent activator of the same system in certain circumstances.

Human serum (1 ml.) was diluted five times and incubated for 2 h at 22° C with 50 U of L-asparaginase (LA). The enzyme was obtained from Merck, Sharpe and Dohme and was dialysed overnight against phosphate buffer (0.005 M) before use. The serum was then diluted a further ten times and its complement (C) activity was measured using 1×10^8 sheep erythrocytes sensitized with rabbit antibody (EA)⁷. The percentage haemolysis was determined kinetically by stopping the reaction with citrate-saline at intervals and measuring absorbance at 415 nm (Table 1). The same procedure was used in the case of guinea-pig serum except that it was diluted

Table 2 Percentage Haemolysis obtained with Guinea-pig Serum

LA added (U)	Min of incubation		
	7.5	10.5	18
None	39.5	81.0	95.0
50	5.0	36.0	80.0

a further 200 times and readings were taken at shorter intervals (Table 2). Both human and guinea-pig sera have less C activity after treatment with LA; after 60 min, this effect is total and irreversible in human serum but partial and reversible in guinea-pig serum.

In a second experiment, the intermediate EAC142 (ref. 8) was prepared by incubating 5×10^8 EA cells in 1 ml. with 0.25 ml. of human R3 for 2 min 30 s at 37° C. The cells were then incubated with ethylenediamine tetraacetate-treated guinea-pig serum (EDTA-serum) diluted thirty times, a reagent having the activity of the terminal C components from C3 to C9. After 25 min, the reaction was stopped by the addition of citrate-saline and the percentage haemolysis was determined by reading absorbance at 415 nm. The results were compared with those obtained with a similar intermediate prepared with an R3 reagent (diluted 1/15) that had been incubated for 15, 60 and 100 min at 22° C with varying amounts of LA (Table 3) before being mixed with EA cells. A significant inhibition of haemolysis occurred in the tubes containing the EAC142 intermediate prepared with an R3 pre-incubated with LA. Moreover, the inhibition seemed to depend on both dose and time.

Table 3 Percentage Haemolysis after Incubation of the R₃ Reagent

Asparaginase added to R ₃ (U)	Min of incubation		
	15	60	100
None	66.5	71.0	75.0
5	70.0	46.0	57.0
10	62.8	18.0	31.0
25	51.0	15.0	6.0
50	27.0	11.0	6.0

In the next experiment, guinea-pig serum was diluted five times and incubated with various quantities of LA. After incubation for 120 min EDTA was added to a final concentration of 0.01 M and the reagent was used to haemolyse EAC142 cells. From the data in Table 4, it can be concluded that LA has no influence on the terminal C factors C3 to C9.

It therefore seems that the inhibitory effect of LA on C takes place early in the sequence of C fixation, at the C1, C4 or C2 steps. Cells in the EAC1 state were prepared by incubating 1×10^8 EA cells with a suitable dilution of a C1 preparation for 10 min at 37° C⁹. The EAC1 intermediate was lysed by guinea-pig serum which had been diluted forty times and treated with Na₂ Mg-EDTA to permit the action of all the C components except C1. EAC1 was prepared in the same fashion, except that the C1 preparation was pre-incubated with 50, 25 and 10 units of LA during the 45 min before contact with EA (Table 5). Surprisingly, unlike the EA cells treated with native C1, the haemolysis of the EAC1 intermediate prepared with LA-treated C1 was greatly increased

Table 1 Percentage Haemolysis obtained with Human Serum

LA added (U)	Min of incubation			
	10	30	50	60
None	3.3	45.0	62.0	67.0
50	0	0	0	0

Table 4 Percentage Haemolysis of EAC142 Intermediate

Asparaginase added to guinea-pig serum (before EDTA) (U)	% haemolysis
None	81.0
50	78.0
25	82.5
10	81.0

Table 5 Percentage Haemolysis of EAC1 Intermediate

LA added to C1 before contact with EA (U)	% haemolysis
None	25.9
50	31.0
25	59.5
10	67.0

after addition of Na₂ Mg-EDTA-serum. Moreover, the smaller the quantity of LA the greater the potentiation of haemolysis.

In other experiments, the use of cells in the EAC4 state enabled us to locate the LA action at the C1 stage. EAC4 cells were prepared after elution of C1 by EDTA from EAC14 cells¹⁰. To three tubes, one containing EAC4 (1×10^8 cells), one a suitable dilution of C1 and one a suitable dilution of C2, 75 U of LA was added. The tubes, together with tubes containing equivalent volumes of EAC14, C1 and C2 without LA, were incubated at 37° C for 60 min. From the pre-incubated samples, various mixtures of EAC4 and C1 were made and incubated for 20 min at 37° C (Table 6). C2 was added after centrifugation. The EAC142 so formed was centrifuged and incubated with guinea-pig serum diluted twenty-five times and treated with EDTA. The results confirm that LA produces its potentiating effect only in the presence of free C1. Whenever C1 is present in free form, LA behaves as a potent activator, the best results being observed with C1 which has been pre-incubated with LA (line 3 of Table 6). Moreover, fixed C4 is unaffected by LA-activated C1.

Table 6 Percentage Haemolysis by EDTA-Serum

Formation of the EAC142 intermediate *	% haemolysis
EAC4 + C1 + C2	10.3
EAC4LA + C1 + C2	40.8
EAC4 + C1LA + C2	51.8
EAC4 + C1 + C2LA	8.0

* The reagents pre-incubated with LA are designated EAC4LA, C1LA, C2LA.

At first glance, the results seem contradictory. Although LA inhibits C in whole serum, it behaves as a potent activator of the same system provided its action is limited to the first step in C fixation, that is, to the formation of cells in the EAC1 state. This can be explained, however, if one assumes that LA activates C1 with the subsequent formation of a potent C1 esterase. This, in turn, would cause an increased destruction of fluid phase C4 and C2^{11,12} leading to a decrease in haemolysis in whole serum. On the other hand, when LA is incubated with C1 which is, after activation, used for the preparation of cells in the state EAC1, the intermediate produced shows a high reactivity. In this case, since the EAC1 cells are centrifuged before the addition of Na₂-mg-EDTA-serum, no free fluid-phase C1 esterase is present in significant amounts to inactivate C4 and C2. This situation is not unlike that in hereditary angioneurotic oedema¹³, in which a decreased concentration of the inhibitor of C1 esterase results in an overactivity of the first component of C and a subsequent fluid phase destruction of C4 and C2.

The observations described here may well have their counterpart *in vivo*. They might, for example, give a clue to the severe anaphylactic reactions noted in some patients on administration of the drug¹⁴. An exploration of the behaviour of C in the blood of leukaemic patients treated with LA might throw some light on the mechanism of action of this enzyme and clarify the role of complement in therapeutic oncolysis. Moreover, human serum seems more sensitive to the action of LA than gp serum, a finding that is of course not unexpected.

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Carboxymethylpachymaran, a New Water Soluble Polysaccharide with Marked Antitumour Activity

We have reported that the polysaccharides lentinan^{1,2} and pachymaran³ strongly inhibit the growth of sarcoma 180 transplanted in mice, and that they could be considered as excellent immuno-accelerators of cell-mediated responses^{4,5}. It is difficult, however, to dissolve them in water and to label them with isotopes, and their use in biological investigations can therefore be troublesome. We have now prepared a water soluble polysaccharide with marked antitumour activity, known as carboxymethylpachymaran ($[\alpha]_D^{20} = -2.8^\circ$; $c=1$ in H₂O; solubility, 6.7 g/100 ml. H₂O at 30° C) which can be labelled at the carboxymethyl residue with ¹⁴C so that its distribution at the cellular and subcellular level can be investigated.

The results of the antitumour assays of carboxymethylpachymaran on sarcoma 180, Ehrlich carcinoma, MM-102 adenocarcinoma⁶, CCM carcinoma⁷ and NTF lymphosarcoma⁸ are shown in Tables 1 and 2.

Antitumour activity was measured by the method described before². A 7 day old ascites tumour was transplanted subcutaneously into the right groin of a mouse. This produced solid tumours at the site of inoculation. Carboxymethylpachymaran was injected intraperitoneally every day for 10 days starting 24 h after tumour transplantation. In assays terminating at the end of 5 weeks, the mice were killed and the tumours were removed and weighed. Tumour inhibition ratios were calculated by comparing the experimental and control mice. The antitumour effects of carboxymethylpachymaran on sarcoma 180 and MM-102 adenocarcinoma were striking, for the tumours regressed completely in many mice. Any direct action of the polysaccharide against tumour cells similar to that manifested by lentinan was not observed, and whereas pretreatment with lentinan⁵ 14-45 days before

Table 1 Antitumour Effect of Carboxymethylpachymaran on Sarcoma 180

Mice	No. of mice	Route	Dose (mg/kg $\times$ 10)	Body weight change (g)	Death/arrived	Average tumour weight (g)	Tumour inhibition ratio (%)	Complete regression
SWM/Ms	10	i.p.	5	+3.6	0/10	0.9	90.1	5/10
	10	i.p.	1	+4.1	0/10	3.9	61.4	0/10
	10	i.p.	0.5	+2.8	0/10	10.5	-7.1	0/10
	10		Control	+4.2	0/10	9.8		0/10
ICR-JCL	10	i.p.	100	+5.4	0/10	1.0	92.3	1/10
	10	i.p.	50	+5.9	0/10	0.5	96.1	8/10
	10	i.p.	25	+5.5	0/10	0.5	95.9	5/10
	10	i.p.	10	+5.1	0/10	1.7	87.1	5/10
	10	i.p.	5	+5.7	0/10	6.1	53.4	1/10
	10		Control	+4.8	0/10	13.0		0/10
ICR-JCL	10	i.p.	25	+7.0	0/10	0.5	95.4	7/10
	10	i.p.	10	+6.0	0/10	2.1	78.7	4/10
	10	i.p.	5	+6.3	0/10	6.1	38.8	1/10
	10		Control	+7.5	0/10	10.0		0/10
ICR-JCL	10	i.p.	10	+2.5	0/10	0.7	93.2	5/10
	10	i.p.	5	+3.1	0/10	4.4	57.7	1/10
	10	i.p.	1	+2.2	0/10	5.7	45.2	0/10
	10		Control	-0.2	2/8	10.4		0/8
Swiss albino	10	i.p.	5	+1.6	2/8	0.1	99.1	6/8
	10		Control	+5.6	0/10	6.4		0/10
	9	i.m.	5	+4.4	0/9	0.2	96.5	7/9
	9	s.c.	5	+5.4	0/9	0.0	99.6	8/9
	9	i.c.	5	+4.2	0/9	0.8	86.3	7/9
	9		Control	+3.6	0/9	4.9		0/9
	10	per os	100	+2.7	0/10	2.9	14.7	0/10
	10		5	+2.5	0/10	3.1	8.8	0/10
	10		Control	+3.1	2/8	3.4		0/8

Table 2 Antitumour Effect of Carboxymethylpachymaran on Ehrlich Carcinoma, MM-102 Adenocarcinoma, CCM Adenocarcinoma and NTF Lymphosarcoma

Tumour	Mice	No. of mice	Dose (mg/kg $\times$ 10)	Body weight change (g)	Death/arrived	Average tumour weight (g)	Tumour inhibition ratio (%)	Complete regression
Ehrlich carcinoma	SWM/Ms	6	5	+0.6	0/6	12.3	0	0/6
		6	Control	+4.5	1/5	12.3		0/5
		6	200	+4.4	0/6	15.4	-6.3	0/6
		6	100	+3.4	0/6	8.7	40.0	0/6
		6	50	+1.5	1/5	13.7	4.8	0/5
		6	Control	+2.7	0/6	14.5		0/6
MM-102 carcinoma	C3H/arima	8	5	+6.5	3/5	2.3	78.9	0/5
		8	Control	-2.3	1/7	10.9		0/7
CCM adenocarcinoma	SWM/Ms	10	5	+2.4	0/10	7.5	36.4	0/10
		10	Control	+1.2	0/10	11.8		0/10
NTF lymphosarcoma	SWM/Ms	10	5	+2.7	1/9	9.0	-2.3	0/9
		10	Control	+3.4	2/8	8.8		0/8

Carboxymethylpachymaran was injected intraperitoneally in every case. The vehicle was distilled water.

tumour transplantation produced a marked effect, pretreatment with carboxymethylpachymaran at this time was ineffective. On the other hand, carboxymethylpachymaran had a pronounced antitumour effect which was, except for oral administration, irrespective of injection route, whereas lentinan was effective only after intraperitoneal injection.

Carboxymethylpachymaran was synthesized from pachymaran³, a β -(1 $\rightarrow$ 3)linear glucan. A suspension of 3 g of pachymaran in 80 ml. isopropanol was mixed with 8 ml. of 30% aqueous sodium hydroxide, and stirred vigorously. Monochloroacetic acid (3.6 g) was then added to the mixture and the reaction was continued for 2 h at 40°-50° C with stirring. The reaction product, carboxymethylpachymaran, was filtered in suction and washed with 70% methanol-acetic acid solution, 80% aqueous methanol, 100% methanol and acetone, successively, and dried *in vacuo*; the yield was 3.3 g. The degree of carboxymethyl substitution in the polysaccharide thus prepared was 0.95 mol per anhydrous glucose unit. ¹⁴C-Labelled carboxymethylpachymaran could be obtained by

incorporating monochloroacetic acid labelled with ¹⁴C in the mixture.

The studies of the mechanisms of action of antitumour polysaccharides on organ, cellular and subcellular level are in progress using the ¹⁴C-labelled carboxymethylpachymaran. Carboxymethylpachymaran with lentinan is worth considering as a useful agent in cancer research and cellular immunology.

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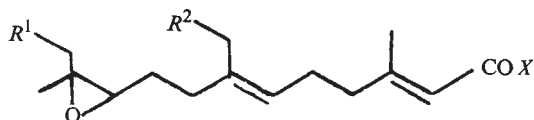
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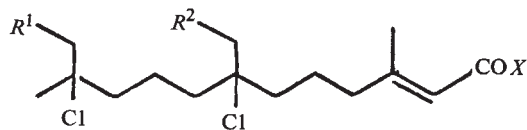
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Terpenoid Amines as Insect Juvenile Hormones

AFTER the discovery that an epoxide derivative of farnesate esters (1) showed high juvenile hormone (JH) activity¹, we undertook to synthesize related compounds in an effort to find the most potent of this class of materials. The elucidation of the structure of two hormones from *Hyalophora cecropia* (2 (ref. 2) and 3 (ref. 3)) as homologues of the farnesate structure, and the observation that products obtained from the addition of hydrogen chloride to farnesate esters (18) also possessed hormone activity⁴, led to the inclusion of analogous compounds in our programme. Here we describe results with a series of amide derivatives.



- | | |
|-------------------------------------|-----------------------------------|
| 1, $R^1=R^2=H$, $X=OCH_3$ | 4-10, $R^1=R^2=H$, $X=NHR^3$ |
| 2, $R^1=R^2=CH_3$, $X=OCH_3$ | 11, $R^1=R^2=CH_3$, $X=NHR^3$ |
| 3, $R^1=CH_3$, $R^2=H$, $X=OCH_3$ | 12-17, $R^1=R^2=H$, $X=N(R^3)_2$ |



- | | |
|-------------------------------|--------------------------------|
| 18, $R^1=R^2=H$, $X=OC_2H_5$ | 20, $R^1=R^2=H$, $X=N(R^3)_2$ |
| 19, $R^1=R^2=H$, $X=NHR^3$ | 21, $R^1=R^2=H$, $X=NHR^3$ |

The method used for the preparation of compounds (5) to (17) involved the phosphonate modification of the Wittig reaction⁵. Condensation of the appropriate N-substituted diethyl carbamoylphosphonate with geranylacetone, using sodium hydride as the condensing agent, gave the olefinic precursors to compounds (5)-(10) and (12)-(17). The precursor to compound (11) was obtained by a similar reaction with 6-ethyl-10-methyl-5,9-dodecadien-2-one⁶. Preparation of the intermediate N-substituted diethyl carbamoylmethylphosphonates involved treating chloroacetyl chloride with the appropriate amine to give N-substituted chloroacetamides, followed by a Michaelis-Arbuzov reaction with triethyl phosphite⁷. The precursor to the unsubstituted amide (4) was prepared by hydrolysis of the corresponding nitrile, obtained in turn from geranylacetone and diethyl cyanomethylphosphonate⁸. Epoxidations were carried out with *m*-chloroperbenzoic acid in

methylene chloride, and the products were purified by chromatography on silica gel.

The dichloro derivatives (19-21) were prepared by adding hydrogen chloride to farnesenic acid or 7-ethyl-3,11-dimethyl-2,6,10-tridecatricarboxylic acid, followed by conversion to the acid chloride with thionyl chloride⁹ and treatment with the appropriate amine. Attempts to prepare the dichloro derivatives of the amides by adding hydrogen chloride to the corresponding olefinic compounds were unsuccessful. All compounds prepared for this study were fully characterized by elemental analysis and by nuclear magnetic resonance and infrared spectrometry.

Table 1 Juvenile Hormone Activity of Terpenoid Amide Derivatives on *Tenebrio molitor*

Compound No.	R^3	Minimum active dose (μ g)	% response
4	H	1.0	70
5	CH_3	0.01	100
6	C_2H_5	1×10^{-4}	100
7	C_3H_7	0.01	63
8	<i>i</i> - C_3H_7	0.1	100
9	C_4H_9	1.0	100
10	<i>i</i> - C_4H_9	1.0	100
11	C_2H_5	1×10^{-5}	70
12	CH_3	1.0	82
13	C_2H_5	0.01	68
14	C_3H_7	1.0	100
15	<i>i</i> - C_3H_7	1.0	100
16	C_4H_9	0.01	65
17	<i>i</i> - C_4H_9	0.1	85
19	C_2H_5	1×10^{-4}	100
19a	C_2H_5	0.01	95
20	C_2H_5	0.01	89
20a	C_2H_5	1.0	100
21	C_2H_5	0.1	43

Activity is minimum dose per pupa required for retention of significant pupal characteristics (equivalent to a "3" on the numerical scale of Bowers and Thompson¹⁰). Compounds are mixtures of all possible isomers except (19a) and (20a).

Initial screening for JH activity was carried out using freshly moulted pupae (10-20 h) of the yellow mealworm, *Tenebrio molitor*¹⁰. Twenty insects were used at each dose level, and minimum active doses were confirmed by three replicates. The substances were applied topically in 1 μ l. of a solvent comprising 9 parts acetone and 1 part oily vehicle, usually squalene.

The presence of the oil in the topical formulations has been found to enhance the activity of many JH agents. This is particularly true of the dichloro derivatives (19-21); in the absence of the oil, these substances were active only at 100 to 1,000-fold larger concentrations. The nature of the oil was also found to be important. In general, oils with high unsaturation gave better results: exceptions were coconut oil and squalene, essentially saturated substances which afforded significant potentiation.

Of the epoxides (5-17), the N-ethyl derivatives (6) and (11) are by far the most potent of the series, and are the most potent in the *Tenebrio* screen of any published JH substances. By comparison, a synthetic mixture of isomers of *Cecropia* JH⁶ had an activity of 0.1 μ g, and 6,7-epoxy-3-ethyl-7-methyl-2-nonenyl-3,4-methylenedioxyphenyl ether¹¹ had an activity of 10^{-3} μ g in the same assay procedure. Among the monoalkyl amides the JH activity falls off rapidly with decreasing and increasing size of the N-substituent. Among the dialkyl-amides both (13) and (16) show significant activity, but do not approach the potency of (6).

Synthesis of the dichloro derivatives (19) and (20) facilitated the isolation of pure *trans* isomers (19a) and (20a). It is interesting to note that in both cases the mixtures (19) and (20) are 100-fold more active than the corresponding *trans* isomers. The mixtures were characterized by nuclear magnetic resonance spectrometry, and were shown to be predominantly the *cis* and *trans* isomers of 7,11-dichloro-7,11-dimethyl-2-dodecenamides. Impurities were detected, however, and they could account for the enhanced activity. The *Cecropia* JH analogue (21) is much less active than the farnesinamide (19); the former is a much more complex mixture of isomers, and has not been as well characterized as (19).

The N-ethyl amides are structurally very similar to the esters (1-3). Increased potencies could well be due to the different binding characteristics of the secondary nitrogen (both an electron pair and a hydrogen atom for noncovalent interactions) or to increased stability of the amide over the ester group. The only other nitrogen containing compounds with JH activity are some carbamates reported by Schwarz *et al.*⁸ and some peptide derivatives reported by Zaoral and Sláma¹².

During our screening programme combinations of JH agents, or JH agents and insecticide synergists, were examined. In most cases there was little, if any, enhancement of activity, but a 1 : 1 combination of (6) and (19) produced a remarkable potentiation in the *Tenebrio* assay. Pupal characteristics were retained by the test insects at doses as low as 10⁻⁶ µg.

Compound (6) is effective in preventing maturation of houseflies (*Musca domestica*) when applied topically to final instar larvae. No normal adults are obtained at doses of 0.01 µg. Compound (19) was less active, affording complete control at 0.1 µg per larvae. Only slight potentiation effects are observed on houseflies with combinations of (6) and (19). Adult mosquitoes (*Culex pipiens*) fail to emerge from pupae when larvae are reared in water containing 0.1 p.p.m. of (6).

Incorporation of either (6) or (19) into the diet of the German cockroach (*Blattella germanica*) effectively controls colony growth and most of the adults die within a few days of being given a bait of starch containing 20 p.p.m. of the active ingredient. Gravid females drop their egg sacs prematurely, and the hatch, if any, is very small. Any nymphs which appear fail to survive to adulthood. The baits have retained their effectiveness for more than 6 months against exposure in a test cage.

Although this effect on cockroaches seems to be a general toxic response, it is also highly specific to this species. Other species, such as *Tenebrio molitor* and *Tribolium confusum*, survive as larvae or adults in media containing up to 100 p.p.m. of either (6) or (19). Development of the larvae is arrested (larvae ultimately die long after pupation would normally have occurred) and eggs laid by the adults fail to develop, but no "toxic" deaths are observed. Other species such as the Indian meal moth (*Plodia interpunctella*) seem to be unaffected by either compound. One can only speculate that the *Corpora allata* hormone(s) have some metabolic function in adult insects, and that the German cockroach is particularly susceptible to disruption of this function.

Acute oral toxicity tests have been carried out with both (6) and (19). Massive doses of (6) (3,200 mg/kg) are without effect on laboratory mice. Compound (19) has an observed LD₅₀ of 3,980 mg/kg, a value which indicates this material is relatively safe as far as acute toxicity is concerned.

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Possible Role of the Pituitary/Adrenocortical Axis in Aggressive Behaviour

THE recent finding by Cherkin and Meinecke¹ that aggressive behaviour in previously isolated male rabbits could be suppressed for some weeks by allowing them to recover in pairs from barbiturate anaesthesia, raises an interesting question concerning the action of components of the pituitary/adrenocortical axis on behavioural responses. It is well established that most forms of anaesthesia are "stressful" in that they result in activation of the adrenal cortex, and the possibility thus exists that findings such as those by Cherkin and Meinecke are a consequence of changes in ACTH.

The role of pituitary and adrenocortical hormones on behaviour has been recently reviewed by Levine², and evidence has been obtained that ACTH depresses isolation-induced aggressive behaviour in the albino mouse^{3,4}.

Defeated animals show a pronounced increase in adrenocortical activity⁵⁻⁷ which is not evident in the victor and it seems likely that this increase in ACTH and/or glucocorticoids may have a cue function in inducing subordinate behaviour by increasing the fear response and reducing the aggressive response. An alternative explanation for the result obtained by Cherkin and Meinecke is thus that the stress of anaesthesia causes both animals to assume subordination on emerging from anaesthesia with a consequent suppression of aggressive behaviour. This subordination could be specific to the animal in whose presence they recovered: dominance could be associated with the visual or olfactory characteristics of the other animal.

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Myoid Conduction in the Siphonophore *Nanomia bijuga*

Myoid conduction has been suspected in the Cnidaria for some sixty years¹, but has only recently been demonstrated^{2,3}. There are no recordings available of activity at the cellular level; until now potentials from cnidarian muscles have been recorded with large extracellular electrodes covering a number of active units.

The stem of the siphonophore *Nanomia bijuga* is covered with a layer of epithelio-muscular cells some 45 μm thick (Fig. 1); this is far thicker than similar cells in many other cnidarians. The muscle fibrils run longitudinally and are non-striated.

Portions of the stem, about 4 cm long, were pinned onto the

wax base of a cooled chamber and covered with seawater maintained at 13° C. Cells were successfully impaled by glass microelectrodes filled with 2.8 M potassium chloride having resistances of between 60 and 80 M Ω . Resting potentials of between -50 mV and -80 mV were obtained with 63 mV being the mean of twenty penetrations. This value is in agreement with the values given for anthomedusan epithelia³. Many preparations showed spontaneous activity with cell interiors depolarizing 17–20 mV (Fig. 2). The rise time of these potentials is approximately 10 ms, but repolarization takes up to 3 s. These events were conducted without decrement along 2 cm of the stem with a conduction velocity of 20 cm/s as measured by a pair of extracellular suction electrodes.

If the preparation was shocked by square wave pulses of 1 ms duration delivered by a suction electrode to the surface of the stem two distinct types of potential changes were seen in the conducting cells. The type of potential change was dependent on stimulus strength.

The electrical constants of this preparation were not known, and so the exact current delivered below the stimulating electrode could not be calculated. With the distance between the microelectrode and the stimulating electrode at 1 cm, shocks delivered at a potential of 3 V gave no change in the potential of the cell interior (Fig. 2). At 4 V there is a distinct depolarization of the cell membrane and at 6 V an even greater change so that the interior of the cell became 4 mV more positive. In such cases the rate of increase of positive charge inside the cells is very slow. Events such as these which I have called sub-threshold pulses are not propagated but are probably conducted with decrement. At a specific stimulus strength (about 9.5 V) for any one preparation the penetrated cell will fire giving a rapid decrease in the potential across the outer membrane of the cell. This event I have called a supra-threshold pulse and it seems to be identical to the spontaneous pulses already described.

Each supra-threshold pulse causes a slight contraction of the longitudinal muscle fibres, often sufficient to dislodge the microelectrode. Contractions during spontaneous firing never caused the microelectrode to come out of the cell. If a micro-

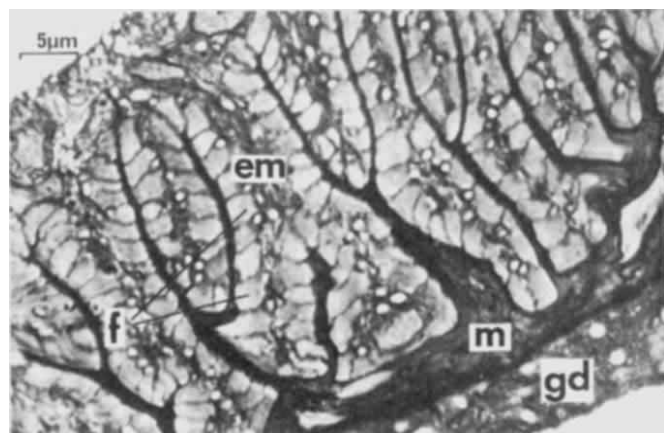


Fig. 1 Transverse section of stem of *Nanomia*, 0.5 μm 'Epon' section (Richardson's stain); em, single layer of epithelio-muscular cells; f, fibrils of epithelio-muscular cells; gd, gastrodermis; m, mesogloea.

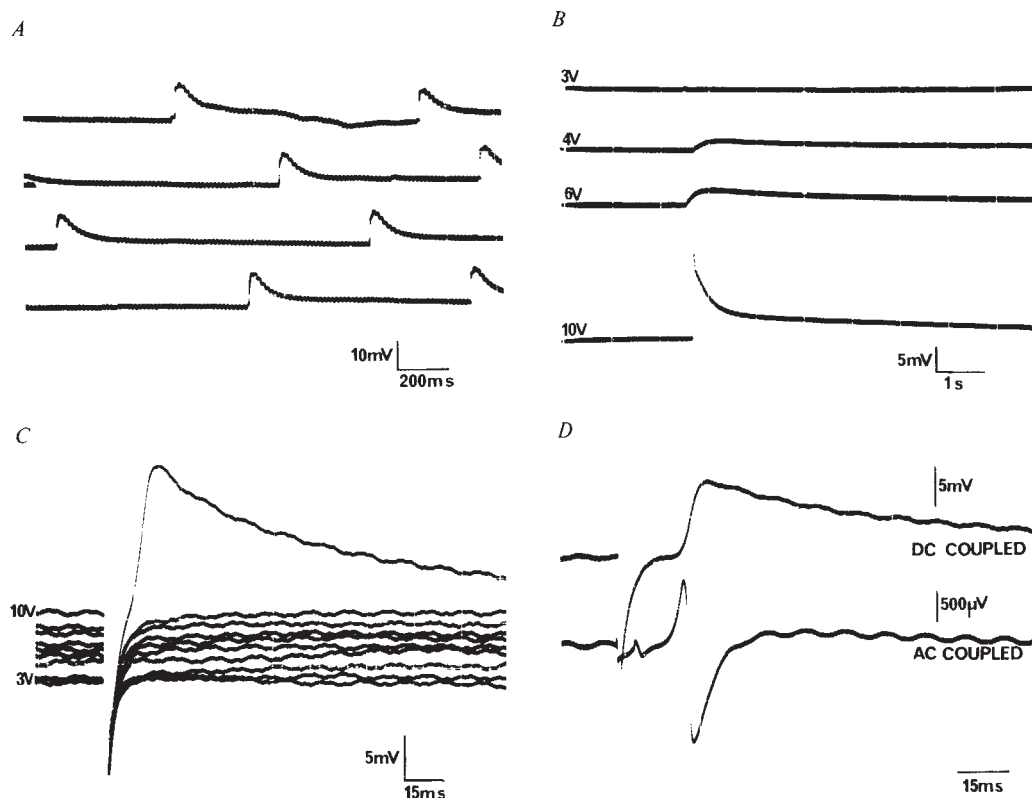


Fig. 2 A, Spontaneous supra-threshold pulses; B, electrically stimulated pulses showing change from sub-threshold pulses to a supra-threshold pulse as stimulus strength is increased; C, same as B but tracings superimposed; D, a stimulated supra-threshold pulse as recorded by an intracellular electrode (upper trace), and an extracellular suction electrode above the intracellular electrode (lower trace).

electrode is placed directly beneath a suction electrode one can compare the passage of a supra-threshold pulse as recorded inside and outside the cell. The suction electrode in this case is covering more than one cell. The wave-shape and duration of this pulse, as recorded by the extracellular electrode, are very similar to those recorded from other hydrozoan epithelia³⁻⁵.

Procion yellow and Niagara sky blue 6B injected iontophoretically diffused through a number of cells and so failed to show the exact location of the electrode tip. Use of a screw-micrometer to advance the electrode indicated that the tip in most cases would have been in or just above the muscle fibrils.

The preparation continued to propagate supra-threshold pulses when immersed in a solution containing one part seawater and one part isotonic magnesium chloride. Excess Mg^{2+} blocks nervous activity in other hydrozoans, so it seems that propagation of supra-threshold pulses does not depend on the nerve-net³.

It is not yet possible to prove that these recordings are intracellular—they could conceivably be transepithelial pulses. Such pulses have been recorded between the enteron and surrounding water in *Hydra* but they have the opposite polarity to the pulses recorded in *Nanomia*⁶. If they are intracellular recordings then one might suppose that the cells in this conducting epithelium are connected by low resistance pathways as demonstrated by the local spread of sub-threshold excitation. This is a reasonable hypothesis since similar types of junctions have already been proposed for conducting epithelia and smooth muscles⁷⁻⁹.

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Corrosion by the Sulphate-reducing Bacteria

THERE are several explanations for the aggravation of corrosion of iron and steel in oxygen-free conditions by sulphate-reducing bacteria. These are (a) stimulation of the cathodic part of the corrosion cell by the removal and utilization of the polarizing hydrogen by the bacteria¹; (b) stimulation of the cathodic reaction by solid ferrous sulphides formed by the reaction of ferrous ions with sulphide ions produced by bacteria²; (c) stimulation of the anodic reaction, metal dissolution, by bacterially produced sulphide³; (d) local acid cell formation⁴,

and (e) formation of iron phosphide by reaction of the metal with bacterially reduced phosphates⁵.

There is little evidence that (d) and (e) are significant except possibly in isolated cases, whereas (c) is probably important only at the start of the corrosion process due to the eventual formation of protective sulphide films in the presence of free sulphide ion⁶.

Much support for (a), the cathodic depolarization mechanism first postulated by von Wolzogen Kühr, has come from work by Booth and Tiller⁷. Using mild steel electrodes at various controlled potentials, they observed a straight line relationship between the hydrogenase activity of the bacteria and the extra current required to maintain these potentials on the cathodes in the presence of resting-cell suspensions of sulphate-reducing bacteria, provided with benzyl viologen as electron acceptor. But the organisms used had been cultured on standard sulphate-containing medium and the resting-cell suspensions would therefore contain ferrous sulphide, both free and embedded in the mucopeptide material adhering to the cells. (The ferrous sulphide that forms during growth in sulphate media is impossible to separate completely from the bacteria except by chemical methods so severe that the bacteria themselves are destroyed.)

Using the same procedure as Booth and Tiller⁷, we have observed a direct relation between the amount of chemically prepared ferrous sulphide added to a bacteria-free system, and the extra current required, as previously described. Moreover, organisms previously grown in a sulphate-free fumarate medium⁸ showed little cathodic depolarization. This latter result could explain the low weight loss/time results obtained by Booth, Elford and Wakerley² with mild steel coupons in semicontinuous cultures of the bacteria in a sulphate-free fumarate medium. The similarity between the results of their potentiostatic experiments and those obtained by Booth and Tiller⁷ suggests that there is some connexion between the rate of bacterial hydrogen uptake and the amount of ferrous sulphide present (this possibility was adumbrated by the observation of Goldner *et al.*⁹ that particulate material stimulated hydrogen uptake). We have now confirmed this, using Warburg manometry to measure the rate of hydrogen uptake by bacteria grown on both sulphate-free and sulphate-containing media. When chemically prepared ferrous sulphide was added to the resting cell suspensions in the reaction flasks, substantially greater rates of hydrogen uptake were observed in nearly every case, using either sulphate or benzyl viologen as electron acceptor (Table 1).

This effect of ferrous sulphide may explain the wide variation encountered in manometric determinations of $-Q_{H_2}$ for the sulphate-reducing bacteria¹⁰, since variable amounts of ferrous sulphide are carried over into the reaction flasks as is easily verifiable visually; the effect may also have a bearing on the observation¹¹ of a small hydrogen uptake by *Desulfotomaculum orientis*, a sulphate reducer usually considered to be hydrogenase-negative, and this question is being studied further.

Ferrous sulphide alone, though known to be cathodic to mild steel^{12,13}, is not a permanent cathode. Our weight loss/time experiments indicate that chemically or bacterially produced ferrous sulphides corrode by a first order reaction and that the eventual total corrosion is directly proportional to the

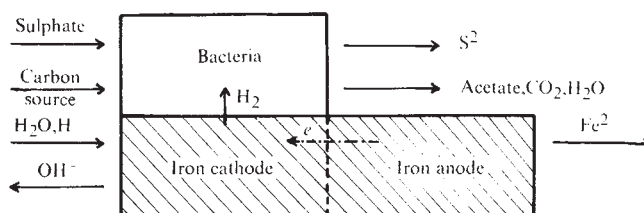


Fig. 1 Scheme illustrating the classic theory of cathodic depolarization by the sulphate-reducing bacteria.

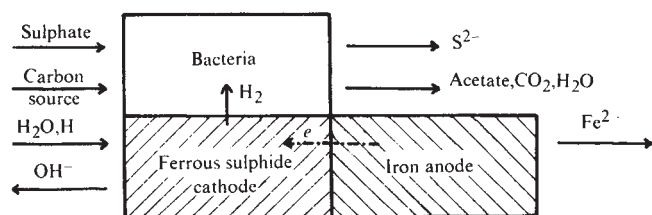
Table 1 Hydrogen Uptake by Sulphate-reducing Bacteria (*Desulfovibrio desulfuricans* Strain Teddington R, NCIB 8312)

Growth medium*	Electron acceptor for manometry	- Q_{H_2} †	
		Resting cells alone	Resting cells with 1 mM ferrous sulphide added
Sulphate-containing lactate medium]	sulphate	30.7	40.6
		43.5	290
		11.3	126
Sulphate-free fumarate medium ⁸	sulphate	6.8	124
		5.6	41
		627	648
	benzyl viologen	443	577

* Bacteria were suspended in 0.04 M phosphate buffer (pH 7.0) for manometry.

† Additions of ferrous ion (as $FeCl_2$) or sulphide ion (as Na_2S) alone did not increase the hydrogen uptake rate. Ferrous sulphide was inactive towards hydrogen for additions of sulphate or benzyl viologen in absence of the bacteria.

amount of ferrous sulphide present. Sustained high corrosion rates in such bacteria-free systems are only obtained by continual replenishment of the ferrous sulphide such as occurs in cultures in iron-rich medium where all the sulphide or bisulphide ion produced by bacterial metabolism is precipitated as ferrous sulphide¹³. In media containing minimal iron, free sulphide ions cause the formation of sulphide films which confer fair protection to the metal underneath. These films are not permanent, however, and the time for breakdown to occur is probably linked to the activity of the bacteria¹³. High rates of corrosion are observed once film breakdown occurs. It seems that once there is sufficient ferrous sulphide present to initiate the formation of the galvanic cell ferrous sulphide/Fe, then a high corrosion rate is attained and no sulphide films are formed. An observation that steel coupons in semicontinuous cultures of sulphate-reducing bacteria, grown initially on iron-rich and later on minimal-iron media, exhibit continued high corrosion rates supports this view (personal communication from D. S. Wakerley).

**Fig. 2** Scheme illustrating the proposed mechanism of corrosion by the sulphate-reducing bacteria.

The presently accepted theory of corrosion by cathodic depolarization is represented diagrammatically in Fig. 1. We propose a new theory of corrosion by the sulphate-reducing bacteria such that the cathodic reaction, hydrogen evolution, occurs on the ferrous sulphide produced by reaction of ferrous ion with bacterially produced sulphide ion. We find that the ferrous sulphide activity is diminished with time, possibly owing to the bonding of atomic hydrogen within the ferrous

sulphide crystals; but the activity of the sulphide is restored by removal of the atomic hydrogen by bacterial hydrogenase activity. This mechanism is illustrated schematically in Fig. 2.

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Infection of Schistosomes with Gram-positive Cocci

Schistosoma spindale (Montgomery) is a common trematode parasite of cattle, water buffalo, goats and other mammals over a vast area including southern Africa, South and South-East Asia. It is a close relative of the human parasite *S. haematobium* and while it has only rarely been reported from man it is a significant producer of cercarial dermatitis in rice field workers. As part of an investigation of *S. spindale* in Malaysia, a number of laboratory animals were infected in various ways with cercariae of this helminth. One young female white mouse was exposed by tail immersion to numerous cercariae of a local strain in Kuala Lumpur and killed 82 days later with 0.3 ml. of heparinized 'Nembutal' (pentobarbital sodium) injected intraperitoneally. The liver and spleen were larger than those usually found in mice with *S. spindale* infections of similar age. The spleen was soft and yellowish-white and particularly distended over about half its length. Small pieces of liver, spleen, lungs and intestine were fixed in 10% formalin, after which the mouse was perfused with citrated saline according to the method of Duvall and DeWitt¹. Eighteen pairs of worms and thirty adult males were recovered in the perfusate; all except one pair and two males had nodular lesions (Fig. 1 b-f) visible to the naked eye or with low magnification. I found several additional worms, including some with such lesions, in the tissues (Fig. 1f), but no affected worms in fifty-seven other mice similarly exposed and perfused. The discovery of these diseased worms was unanticipated. Because their significance was not immediately appreciated, they were fixed with 10% formalin and stored in 70% alcohol as usual. On subsequent examination, it became clear that their nodular lesions were filled with great

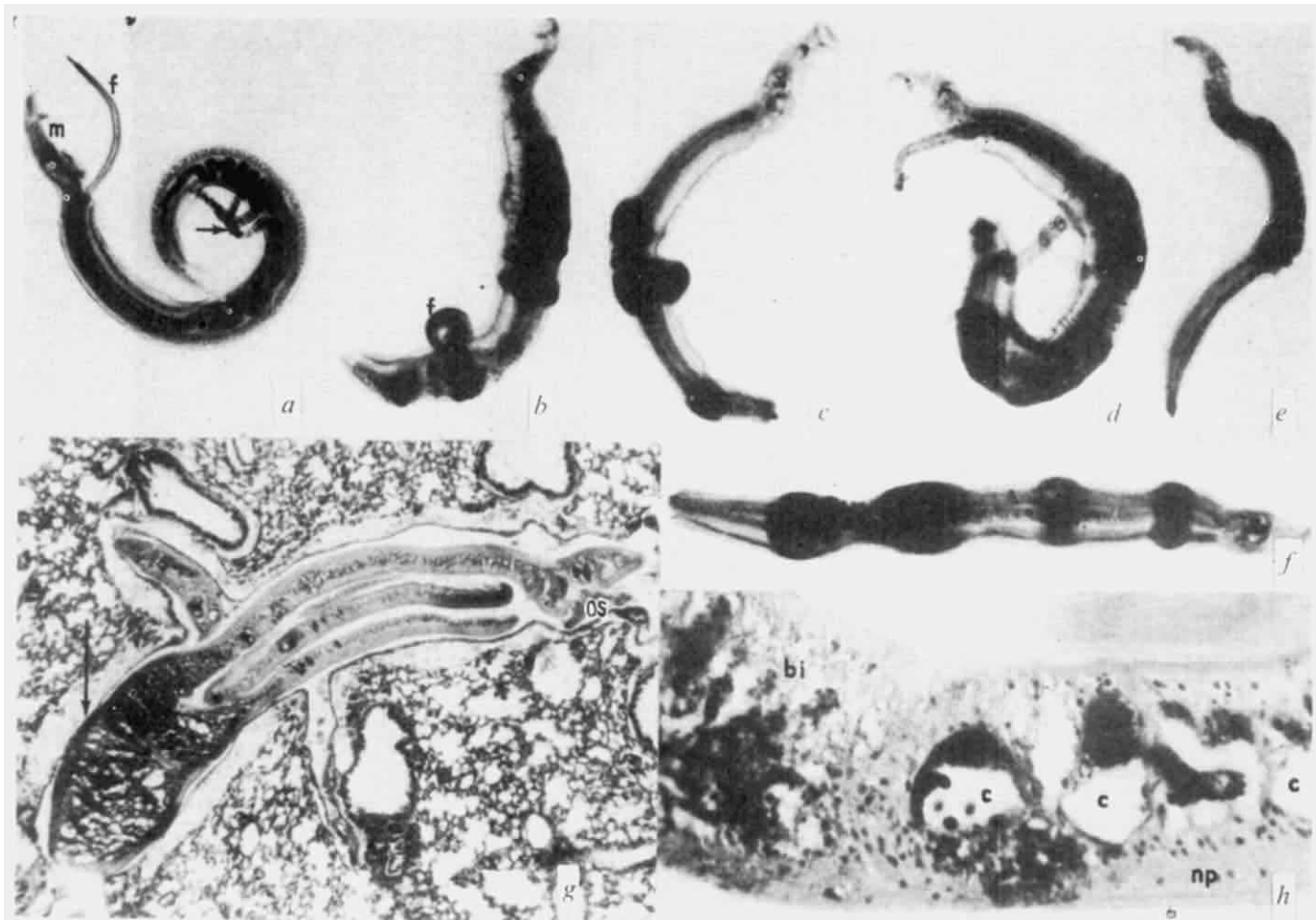


Fig. 1 Bacterial invasion of adult *Schistosoma spindale*. *a*, Relatively normal pair of worms. Note slight thickening in the female (arrow). *b-f*, Worms and worm pairs showing lesions due to bacterial multiplication. *g*, Section of mouse lung showing anterior part of male worm with severely affected region full of bacteria (arrow). *h*, Section of isolated male perfused from the portal vein, showing area of bacterial invasion. bi, Bacterial invasion; c, gut caecum; f, female; m, male; np, normal parenchyma; os, oral sucker.

numbers of cocci (Fig 1g and h) which could by then be identified only as being Gram-positive.

The mouse spleen, after paraffin embedding, sectioning at 8 μ m, and staining with haematoxylin and eosin, showed many clumps of cocci in the centre of the expanded whitish region in areas of necrosis and intense cellular activity. Many polymorphonuclear leucocytes were present, together with macrophages containing phagocytosed bacteria. Numerous fibroblasts were seen in and around the region. The condition was consistent with acute splenitis of several days' duration. Bacterial invasion of the spleen was perhaps facilitated because that organ was already compromised owing to portal hypertension induced by the schistosome infection. In the liver, a small area of bacterial invasion surrounded the remains of a worm whose caeca contained masses of bacteria.

It is likely that bacteraemia in the mouse led to infection of the schistosomes. The worms, which dwell in the intestinal and portal venous systems, had probably ingested bacteria along with their usual diet of blood. Sections and stained whole mounts of worms show that multiplication of bacteria occurs primarily in the gut caeca, which become greatly dilated; the parenchyma is then subject to massive invasion (Fig. 1h). The worms, in turn, act as amplifiers of the bacterial infection and serve as intense focal sources of organisms for potential invasion of adjacent tissues. Krakower *et al.*² found that Puerto Rican *S. mansoni* in some rats deficient in vitamin A were invaded by Gram-positive bacilli such as *Lactobacillus acidophilus*, a normal gut inhabitant presumed to have passed through the intestinal wall as a result of the malnutrition.

Ottens and Dickerson³ attempted to cure schistosome infections in mice, hamsters and monkeys by injecting bacteria into the host animal. They found *S. mansoni* to be highly susceptible to certain strains of a variety of Gram-negative bacilli and also observed bacterial invasion and lethal effects in *S. mattheei*, a member of the "haematobium" group to which *S. spindale* also belongs. These authors, moreover, evaluated twenty-four strains of Gram-positive bacteria (not specified), none of which invaded schistosomes.

This report is the first to demonstrate that Gram-positive cocci may be highly invasive and pathogenic in adult schistosome worms.

This work was done in part when I was a staff member of the University of California ICMRT at the Institute for Medical Research, Kuala Lumpur, Malaysia; supported by a US Public Health Service grant. I thank Drs S. DeWitt and K. Vosti of Stanford University for their comments.

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Potential Cocarcinogenicity of Sodium Hypochlorite

SODIUM hypochlorite has been widely used as a bleaching agent and disinfectant. No particular hazard of this chemical to man has been recognized (see, for example, ref. 1) except for the poisoning of infants by the accidental ingestion of concentrated solution. We now report, however, that sodium hypochlorite is definitely concerned in cancer production in mice as a cocarcinogen²⁻⁴.

In testing for cocarcinogenicity, a group of animals is treated with a potent carcinogen in a dose which by itself is not enough to produce a cancer. A suspected chemical cocarcinogen is also given to the same animals, either before or after. The development of a cancer offers a sensitive test for potentially carcinogenic compounds⁵⁻⁷.

A commercial sodium hypochlorite solution which contained more than 10% effective chlorine was used. The carcinogen selected was 4-nitroquinoline 1-oxide^{8,9}. The mice were ddN strain females maintained on water and a standard pellet diet. Before the application of the chemicals, hair was clipped off over an area 1 cm in diameter in the interscapular region where the chemicals were applied.

Three groups of forty 5 week old mice were used. One group was used in a combination experiment.

Twenty applications of 4-nitroquinoline 1-oxide alone produced neither malignant nor benign tumours. Although hypochlorite did not induce skin tumours by itself in sixty applications, skin tumours were induced in nine of thirty-two mice by forty-five applications given after submanifestational doses of 4-nitroquinoline 1-oxide. Thus, one fibrosarcoma of the skin and three squamous cell carcinomas were produced after about 240 days. Five papillomas remained until the end of the experiment. In one tumour-bearing mouse metastases to lung and kidney were observed. Besides the skin tumours, one lymphatic leukaemia was found in another mouse about 360 days after the start of the experiment. No tumours resulted from sixty paintings of aqueous sodium hydroxide at pH 11 ~ 12 combined with twenty applications of 4-nitroquinoline 1-oxide, indicating that potential carcinogenicity of hypochlorite solution is due to the hypochlorite itself and not to the alkalinity of the solution.

Solutions of sodium hypochlorite containing approximately 5% chlorine are available commercially and used widely. For bleaching, the solution is diluted up to several hundred times. Various materials, including foods, may be sterilized using a solution diluted several hundred times. Drinking water may be disinfected by a low concentration of chlorine (0.1 to several parts per million). In the treatment of some skin diseases caused by fungi, a dilute solution may be applied directly to the diseased part. Although in such conditions it seems unlikely that this common chemical constitutes a practical carcinogenic hazard, the data presented here provide a caution against its indiscriminate use.

We thank Dr W. Nakahara, director of the National Cancer Center Research Institute, for valuable discussion and pathological diagnosis and Professor T. Ukita of the Faculty of

Table 1 Carcinogenicity of Sodium Hypochlorite in Mice when Combined with a Submanifestational Dose of Carcinogenic 4-Nitroquinoline 1-Oxide

	No. of mice	Skin tumours		Leukaemia
		Malignant (No. of mice)	Benign (No. of mice)	
Group 1	32	4	5 (1)	1
Group 2	29	0	0	0
Controls	27	0	0	0

The potent carcinogen was first painted on in twenty applications of 0.05 mg in 0.25% (w/v) benzene solution⁵ in the course of 50 days (1.0 mg/mouse in total). Six days after the end of this treatment, one drop (about 0.05 ml.) of the hypochlorite solution was painted on and repeated forty-five times during 245 days (group 1). Other mice (group 2) were treated only with 4-nitroquinoline 1-oxide. The control mice were treated only with the hypochlorite (sixty times in 300 days). All of the mice treated were observed for 450 days from the start of the experiment.

The number in the parentheses shows the number of papillomas that regressed.

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Hormonal Pregnancy Tests and Neural Tube Malformations

IN 1967 Gal, Kirman and Stern¹ reported a possible relationship between pregnancy tests using pills containing synthetic oestrogenic and progestogenic hormones and the birth of a child with spina bifida. Of the 100 mothers of children with spina bifida, nineteen gave a history of pregnancy diagnosis by this means; only four of the 100 matched control mothers gave such a history.

A similar retrospective enquiry has been made between 1968 and 1970 in three centres, but including births of the clearly related malformation anencephaly as well as spina bifida. In the London series the control mothers were those having the next baby born in the same hospital. In the Exeter series the control mothers were matched for area of birth,

Table 1 Anencephaly and Spina Bifida in Babies Born to Mothers who had had Hormonal Pregnancy Tests

		Survey cases		Controls	
		Total	Pregnancy tests	Total	Pregnancy tests
South Wales	Spina bifida	29	2	112	7
	Anencephaly	31	2		
Exeter	Spina bifida	45	3	64	7
	Anencephaly	19	3		
London	Spina bifida	75	4	75	2
	Anencephaly	72	8		
Total		271	22 (20.1)	323	22 (23.9)

The number expected in survey and control groups if no difference exists is shown in parentheses. The difference between actual and expected numbers is within the standard error.

parity and month of conception. In the South Wales series the control mothers were those who had one baby with spina bifida or anencephaly and had a subsequent normal birth during the survey period and these were thus not controlled individually. The findings are summarized in Table 1. In these three surveys there is no significant association of pregnancy tests with pills containing oestrogenic and progesterogenic hormones and the birth of children with neural tube malformations.

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Effect of *Avena sativa* on Cigarette Smoking

IN 1967, in India, I came across a practitioner of ancient Ayurvedic medicine who successfully used a decoction of common oats (*Avena sativa*) to cure the opium habit. While using an alcoholic extract of the plant on a group of opium addicts, several patients reported a loss of interest in smoking. The drug is listed in the United States Dispensatory and National Formulary¹⁻², yet no reference has been traced regarding its application on smokers (Library of the Pharmaceutical Society of Great Britain, personal communication). I have therefore studied the effect of this drug on a group of smokers.

The active drug was an extract of healthy, fresh plant *Avena sativa* selected just before harvest. I used 1.5 parts of the crushed whole plant by weight in 5 parts by volume of 90% ethyl-alcohol, kept at room temperature with frequent agitation for 72 h and then filtered. The active constituent has not been identified.

Twenty-six cigarette smokers including healthy volunteers and chronic patients in the chest wards of Ruchill Hospital,

Glasgow, including tuberculous patients, participated in the trial. The total duration of their smoking and the average number of cigarettes smoked per day in the preceding six months were recorded.

They were told that a drug was being tested which might affect their smoking, and that they were not to make any conscious effort to alter their smoking during the trial.

Table 1 Numbers of Cigarettes Smoked during Trial

Group I (drug)		Group II (placebo)	
Cigarettes per day before trial	Cigarettes per day after trial	Cigarettes per day before trial	Cigarettes per day after trial
20	20	22	19
25	10	30	28
10	0	18	18
12	0	14	14
11	0	18	17
9	0	17	18
35	3	17	18
25	7	8	18
22	0	18	18
20	7	20	18
25	7	15	14
20	10	10	9
20	10	8	8
Av.	19.5	5.7	16.5
			16.7

Each patient kept a daily record of cigarettes smoked, commenting on any changes in the craving for cigarettes. By random allocation, thirteen patients received the drug and the others received placebo for 28 days. The alcoholic extract (1 ml.) was diluted to 5 ml. and each oral dose was 5 ml. of this dilution given four times a day. No psychotherapy was used. No patients were taking any other drugs which could affect smoking. The patients in both groups were comparable in age, sex and smoking history.

The results of the trial are given in Table 1. In the drug group the total daily consumption by thirteen patients was 254 cigarettes; at the end of the trial it was seventy-four. Five had stopped smoking, seven had reduced it to less than 50% and in one no change had occurred. In the placebo group the total daily consumption at the start was 215, at the end it was 217. Smoking had been stopped by none, reduced to above 50% by six and increased by three; four reported no change.

The two groups were comparable in their smoking habits, since group I "before" (mean 19.5 ± 2.0 s.e.) is not statistically separable from group II "before" (mean 16.5 ± 1.7 s.e.); $t=1.33$, $0.30 > P > 0.20$. Group I "before" is significantly different from group I "after" (mean 5.7 ± 2.0 s.e.); $t=5.21$, $P < 0.001$. Group II "before" is not different from group II "after" (mean 16.9 ± 1.4 s.e.); $t=0.180$, $0.90 > P > 0.80$. The mean differences between the groups due to treatment (group I "before"–group I "after") and (group II "before"–group II "after") are naturally equally significant; $t=5.73$, $P < 0.001$. All these calculations are based on Student's t test.

In the drug group various degrees of loss of craving for cigarettes were reported. The drug seems to reduce the number of cigarettes smoked per day, along with diminished craving for smoking. Moreover, the reduction in smoking seems to continue even 2 months after the termination of the drug. The drug has never been used in dealing with the problem of smoking and, as this was the first instance of its use in smokers, its role and significance are worthy of further investigation.

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² National Formulary of the United States, seventh ed., 60 (1942).

Possible Relationship between Smoking and Peptic Ulcer

EPIDEMIOLOGICAL studies have shown that the incidence of peptic ulcer is higher among smokers than non-smokers¹. No reason for this association has been found. Since personality factors seem to play an important role in this disease, it could be argued that both the development of an ulcer and the habit of smoking heavily result from the same psychological traits and are therefore not related. Recently, however, Jacobson *et al.* demonstrated that nicotine markedly reduced secretion of water and bicarbonate from the pancreas by inhibiting the stimulating effect of secretin^{2,3}. Since gastric acid is normally neutralized in the duodenum by pancreatic juice and, to a lesser extent, by bile, it was thought that nicotine might predispose to ulcer formation by preventing such neutralization. We have tested this possibility in rats treated with a combination of two gastric secretagogues (substances which stimulate secretion of gastric juice), pentagastrin and carbachol, which had been found to produce duodenal ulcers⁴. We have reported that when these secretagogues were administered in small doses known to produce an ulcer incidence of only 36%, the addition of nicotine raised the incidence to 90%, and the ulcers were multiple and very severe⁵. Nicotine, given alone, was without effect. This result indicated that nicotine sensitized the duodenum to the ulcerogenic effects of the two secretagogues used.

Subsequent studies demonstrated that the duodeno-ulcerogenic property of pentagastrin plus carbachol was due to the marked increased secretion of gastric juice elicited by these secretagogues⁶. We therefore investigated whether the pro-ulcer effect of nicotine was due to increased sensitivity of the duodenum to acid itself. We thought that since nicotine inhibits secretion of alkaline pancreatic juice, acid, perfused through the stomach, would not be neutralized and therefore should lead to the formation of duodenal ulcer.

A solution of hydrochloric acid was perfused through the oesophagus of rats, in amounts previously found to produce duodenal ulcers in about one-third of the animals¹. Female Upjohn rats (derived from Sprague-Dawley strain), of an average weight of 220 g, were fasted overnight. On the following morning, a tygon tubing (22 gauge) was inserted, under ether anaesthesia, into the cervical oesophagus through a small skin incision. The tip of the tubing lay 0.5–1.0 cm above the cardiac opening of the oesophagus. On recovery from anaesthesia, the tubing was connected to a syringe mounted on a constant infusion machine, containing either HCl 0.12 N (groups I and II) or water (group III). Acid and water were perfused at a rate of 3.0 ml/h for the first 10 h, then at a rate of 1.5 ml/h for the next 14 h. The fluid flowed from the oesophagus to the stomach, then entered the duodenum through the pylorus. Group I (sixteen rats) was also infused subcutaneously with saline at the rate of 0.54 ml/h, whereas group II (fourteen rats) was infused subcutaneously with a solution

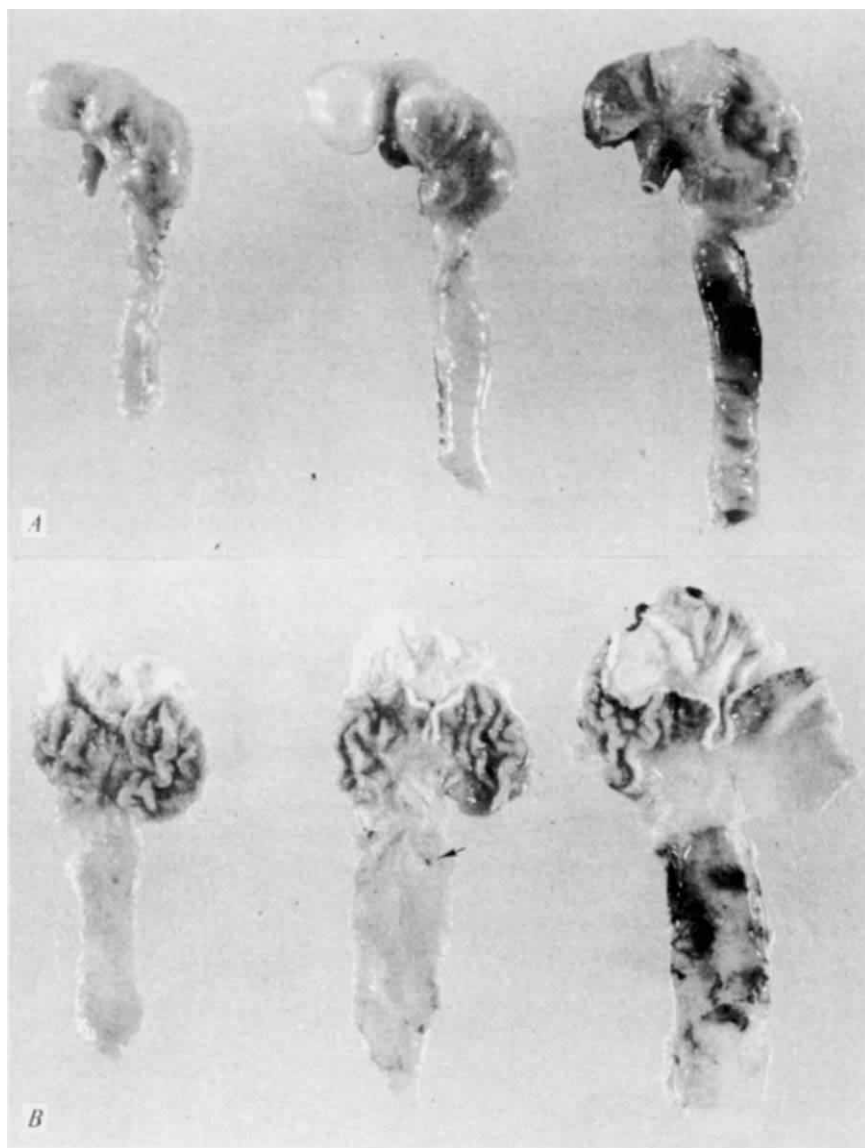


Fig. 1 Duodenal ulcers produced by acid (perfused in oesophagus) and nicotine (infused subcutaneously). *A*, External view of stomach and duodenum. *B*, Same stomachs and duodena after opening along mesenteric attachment (duodenum) and greater curvature (stomach). Left, water perfused in oesophagus, nicotine SO_4 infused subcutaneously (group III). No ulcer. Centre, acid (HCl 0.12 N) perfused in oesophagus, saline infused subcutaneously (group I). Small duodenal erosion (arrow). Right, acid (HCl 0.12 N) perfused in oesophagus, nicotine SO_4 infused subcutaneously (group II). Extensive, deep and multiple duodenal ulcers. Note that the gastric mucosa is intact.

of 20 µg/kg/min of nicotine sulphate in saline also at the rate of 0.54 ml./h. Group III (twelve rats) received the same amount of nicotine sulphate subcutaneously as group II, but water instead of acid was perfused through the oesophagus. During treatment, the animals were conscious at all times and placed in a cylindrical cage that prevented them from bending and thus from chewing the tubing. Food and water were withheld throughout the experiment. All rats were killed after 24 h of treatment. The stomach and duodenum were dissected out as a single unit and opened along the mesenteric attachment for the duodenum and the greater curvature for the stomach. They were then examined with a ×2 binocular magnifier for the presence of ulcerations.

Table 1 shows that when acid alone was perfused (group I), duodenal ulcers were found in 31% of animals. When nicotine sulphate was also given (group II), the incidence reached 93%. The ulcers in the animals treated with acid and nicotine were also more numerous and more severe than those in animals receiving acid only. Finally, nicotine alone (group III) produced no ulcer. Fig. 1 shows duodenal ulcers produced by acid in the oesophagus plus nicotine, administered subcutaneously.

Table 1 Sensitization by Nicotine to Duodenal Ulcers produced by Acid Perfusion

Group	I Acid perfused * + saline s.c.	II Acid perfused + nicotine SO ₄ † s.c.	III Water perfused + nicotine SO ₄ s.c.
No. of animals	16	14	12
Duodenal ulcers			
Incidence %	31	93	0
Severity 0-3+	0.3	1.5	
Ulcers/duodenum	0.4	1.6	
Ulcer index ‡	3.8	12.4	0

Animals were killed after 24 h

* Acid: HCl 0.12 N perfused through the oesophagus

† Nicotine SO₄: 20 µg/kg/min infused subcutaneously.

‡ Ulcer index: sum of (a) % incidence (divided by 10), (b) severity in pulses and (c) numbers of ulcers per duodenum.

These results strengthen the hypothesis that nicotine sensitizes the duodenum to the ulcerogenic property of acid flowing from the stomach. Although, in the present study, the acid was from an exogenous source, the acid concentration and the perfusion rate were close to the amount of secretion formed by the rat stomach when maximally stimulated with histamine⁶. Thus, acid alone can initiate a duodenal ulcer provided either the concentration and rate of flow are high enough (as shown by previous studies⁷), or the formation of alkaline juice from the pancreas is reduced by an agent like nicotine.

The amount of nicotine in one cigarette is about 15–30 mg. Approximately 50% of cigarette smoke can be expected to be inhaled by a heavy smoker and it has been estimated that 90% of nicotine in inhaled smoke is absorbed into the circulation⁹. If man reacts to nicotine as the rat does, it seems likely that nicotine absorbed chronically through smoking may contribute to ulcer formation and to ulcer recurrences by reducing secretion of alkaline pancreatic juice via inhibition of secretin.

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Orientation-specific Masking of Letter Features

THE ability to detect or resolve a target consisting of a briefly presented bar or grating is diminished by preceding or following it with a grating of similar orientation. This orientation-specific masking is maximal when target and mask have the same orientation and the impairing effect of the mask decreases monotonically with increases in the angular difference between target and mask, reaching half its peak value at a difference of about ±15° (refs. 1–4). These findings have generally been attributed to the properties of single cortical cells which seem to act as orientation analysers and which have been shown to possess similar angular bandwidth tuning^{5–7}. Such selective masking may be used to compile pattern feature lists and to elucidate their organization in the recognition of complex patterns⁸. But we know of no studies of selective masking of multi-attribute patterns. Accordingly, we have explored the feasibility of selectively masking orientational features of letters to produce predictable confusions in a typical pattern recognition task.

The recognition stimuli were eight upper case English letters: —A, Z, —Y, N, —H, T, —O, X. A backward masking paradigm was used whereby the letters were presented singly in field 1 of a Gerbrandt three-channel tachistoscope followed after a delay by a masking grating in field 2. The letters were Letraset Futura Bold (96 point) transferred to white cards. At the viewing distance of 580 mm they subtended an angular height of 2° 31'. The component bar width of the letters subtended 40'. They appeared as high contrast black letters on a white background with a luminance of 48 mLamberts and were exposed for 2 ms.

The mask formed a 10° 55' square surrounding the location where the letter had appeared. This square was filled with a black and white square-wave grating with an angular stripe width of 12'. Mask duration was fixed at 55 ms. In the two experimental conditions the orientation of this grating was either horizontal or vertical.

A delay of mask from stimulus offset was chosen for each subject which yielded approximately 60% recognition of the letters with the vertical mask, after some practice. These delay values ranged from 12–37 ms between subjects.

Eight subjects made forced choice recognition responses to the block randomized letters with each letter occurring six times in each condition. A preliminary analysis of the data in Table 2 reveals that the letters which were least well recognized were those with components of the same orientation as the masking grating. Consider the letters Z and N which have the same form subject to a 90° rotation. With a vertical mask Z is better recognized than N, whereas N is markedly better recognized than Z with a horizontal mask. The only anomalous letter is A which is not masked by the horizontal grating. This letter, however, is unique among those studied in that even if its horizontal component is removed the residual inverted V is sufficient for correct recognition. Furthermore, this oblique

feature may fall within the masking angular bandwidth of a vertical grating, as we discuss later.

The crucial test of orientation specific masking is in the nature of the confusional errors. Considering first the interaction of horizontal letter components with the horizontal mask the overwhelming proportion of errors to T is Y, the only other letter with a single vertical feature. Similarly, H is most often confused with N, the best solution for the residual two verticals, and Z with X.

When H and T are masked by the vertical grating, however, both are most often confused with Z, a solution compatible with a residual horizontal component. Y with its vertical stem masked is most often confused with X, and N with X and Y. It seems, therefore, that the pattern of confusions changes according to the orientation of the mask, and these changes are consistent with the assumption that orientation-specific masking of letter components takes place with the response determined by the best fit to the residual unmasked components.

Table 1 Percentage of Errors in Letter Recognition as a Function of Mask Orientation

Subject	Vertical mask Error %	Horizontal mask
MI	14.6	2.1
ST	37.5	10.4
PH	27.0	10.4
LE	47.9	18.8
JI	60.4	22.9
MA	41.7	29.2
JA	22.9	6.3
GA	41.7	4.2
X	36.7	13.0

Errors are totalled over letters.

The vertical grating was a more effective mask for every subject (Table 1). For all but one subject the overall increase in errors due to the vertical mask exceeds 250%. Furthermore, for each target letter the error total across subjects is greater in the vertical mask condition, except for the letter O which was always correctly recognized (Table 2). Because orientation-specific masking holds over a range of angular similarities, and the oblique components of the letters fall nearer to the vertical, they might be more vulnerable to the vertical mask. But with the exception of letter A which has obliques 22° off vertical and which was peculiarly vulnerable to the vertical mask, the oblique components were 32°–38° from the vertical. At this separation masking can be expected to be negligible¹⁻⁴. Furthermore, errors to the letter T increase 50% with the

Table 2 Confusion Matrix of Errors to Letters Masked by Vertical and Horizontal Gratings

Mask orientation	Target letter	Error type and frequency	Error total	Error %
Vertical	A	11X · 3Z · 3H · 1N	18	37.5
	Z	3T · 2A · 2X · 2Y · 2O · 2N	13	27.0
	Y	5X · 3A · 2T · 2N · 2Z	14	29.2
	N	7X · 7Y · 4A · 3T · 2Z	23	47.9
	H	9Z · 7Y · 7N · 5T · 3A · 2X	33	68.8
	T	8Z · 6A · 6Y · 4N · 2H · 1X	27	56.3
	O		0	0
	X	5Z · 3Y · 2H · 2T · 1A	13	27.0
	A		0	0
	Z	5X · 2Y · 2A · 1N · 1H	11	22.9
Horizontal	Y	2X · 1Z · 1H	4	8.3
	N	2H · 1X · 1A	4	8.3
	H	10N · 1O	11	22.9
	T	16Y · 1H · 1O	18	37.5
	O		0	0
	X	3A	3	6.3

Erroneous responses are listed in descending order of frequencies for each target letter. Data are totals across eight subjects.

vertical mask although this letter contains only one vertical and one horizontal component. The greater efficacy of the vertical mask is therefore not explicable solely in terms of the particular orientation characteristics of the letters used. Letters are usually encountered in horizontal arrays, however, and it may be that there are orientational aspects of letter processing other than the orientation of components.

We used a grating frequency of 2.5 c/degree, but further manipulation revealed that masking decreased steeply with increases in spatial frequency over an investigated range of 1.25 to 20 c/degree. Two explanations for this suggest themselves. Apparent contrast varies inversely with spatial frequency⁹ and it seems plausible that apparent contrast determines the efficacy of a masking grating. Second, as grating frequency decreased it approached the value of the bar width of the letters. Thus spatial frequency specific masking¹⁰ could be expected to increase, providing another source of selective masking.

A further implication of these results is that the masks used to terminate the visual persistence of letter arrays may require critical attention. Such "noise" masks seldom have the distribution of spatial frequencies and orientations which is desirable for feature masking. The controversy between integration-degradation and interruption-erasure theories of masking may well depend on the choice of mask. The common choice of a mask in terms of its efficacy when superimposed on the target may maximize integrative masking but will be irrelevant to feature masking if the results of Campbell and Kulikowski³ can be generalized.

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Organochlorine Pesticide Residues in Harbour Porpoises from the Bay of Fundy Region

ORGANOCHLORINE pesticide residues, together with polychlorinated biphenyl compounds of industrial origin, occur in many forms of life in the marine ecosystem. These compounds are concentrated through progressive trophic levels until relatively high levels accumulate in lipid-rich tissues of predatory seals, toothed whales and sea birds¹⁻³.

The highest levels in marine mammal tissues published so far were in the blubber of ringed seals, *Pusa hispida*, from the

Table 1 Organochlorine Residue Levels from Blubber and Liver of Porpoises (*Phocoena phocoena*) from New Brunswick and Nova Scotia (1969, 1970)

Sex status	Sample size	Tissue	Average residue levels in p.p.m. in extractable fat					Σ DDT
			Dieldrin	<i>p,p'</i> -DDE	<i>p,p'</i> -DDD	<i>o,p'</i> -DDT	<i>p,p'</i> -DDT	
Weaned and adult males	12	Blubber	9.24 (4.4–13.1)	121.57 (45.7–181.0)	48.16 (27.4–100.0)	17.84 (9.6–26.7)	119.18 (60.6–175.0)	306.74 (150.8–520.0)
	3	Liver	0.09 (0.01–0.15)	1.37 (0.92–1.61)	0.98 (0.86–1.20)	0.03 (0.01–0.04)	0.35 (0.18–0.47)	2.69 (1.97–3.32)
Male sucklings	2	Blubber	5.30 (0.6–10.0)	54.55 (36.4–72.7)	18.50 (12.0–25.0)	7.60 (1.2–14.0)	50.25 (25.5–75.0)	130.90 (75.1–186.7)
Female suckling	1	Blubber	5.00	72.90	30.00	5.00	46.90	154.80
Immature and resting females	15	Blubber	7.75 (3.0–12.5)	76.94 (36.9–150.0)	39.18 (18.3–80.0)	13.10 (5.3–24.0)	85.04 (40.0–200.0)	214.27 (111.6–447.9)
	8	Liver	0.06 (0.02–0.13)	0.50 (0.25–1.28)	0.46 (0.20–0.93)	0.02 (0.01–0.08)	0.37 (0.10–1.28)	1.36 (0.61–3.57)
Pregnant and/or lactating females	6	Blubber	1.37 (0.1–2.4)	25.50 (15.0–40.0)	13.47 (7.2–24.0)	2.41 (0.1–8.0)	27.75 (14.1–50.0)	69.03 (40.0–122.0)
	2	Liver	0.02	0.26 (0.25–0.27)	0.14 (0.12–0.16)	0.01	0.07 (0.06–0.08)	0.48 (0.46–0.50)

Gulf of Bothnia (Σ DDT range 11–130 p.p.m., mean 120 p.p.m.), and in the blubber of grey seals from the Baltic Sea (Σ DDT range 97–310 p.p.m., mean 170 p.p.m.)². One porpoise, *Phocoena phocoena*, from the east coast of Scotland contained a total residue concentration (dieldrin + DDT compounds) of 73.3 p.p.m.⁴.

Sixty harbour porpoises, *Phocoena phocoena*, were collected by D. E. G. in western North Atlantic waters in 1969–70. Blubber samples from thirty and liver samples from twelve animals were chilled to sea water temperature (about 10° C) and frozen to –20° C as soon as field conditions permitted (2–7 h). A two column fractionation separated organochlorine insecticides from the polychlorinated biphenyls (PCBs). The first step consisted of a 'Florasil' column for the separation of fatty coextractives from organochlorine insecticides⁵. Up to 10 µg PCBs or 1 µg of DDT, DDE, or DDD were handled by the column to give a 90 to 100% separation. For best separation, two elutions with quantities most favourable to each group of compounds were made. Eluates were concentrated almost to dryness and redissolved in hexane for gas chromatography. Qualitative residue confirmation was achieved principally with thin-layer chromatography. Percentage recoveries were checked periodically by fortification directly into the oil obtained from hexane extraction. Average recoveries were: *p,p'*-DDE, 98%; *o,p'*-DDT, 91%; *p,p'*-DDT, 90%; *p,p'*-DDD, 95%; dieldrin, 89%. Data in Table 1 are not corrected for percentage recovery. Further study of the PCB residues is in progress. Their occurrence was, however, quite high, ranging from one to three times the levels recorded for *p,p'*-DDE.

Average dieldrin, Σ DDT levels in pregnant and lactating females were much less (1.37 p.p.m. of dieldrin, 69.03 p.p.m. Σ DDT) than in immature and resting females' blubber (7.75 p.p.m. dieldrin, 214.27 p.p.m. Σ DDT). Three sucklings had high levels (range 75.1–186.7; mean 138.8 p.p.m. Σ DDT). Residues may move into the depot fat in the foetus, or to the neonate through milk, or both.

The average Σ DDT level in the blubber of males (306.74 p.p.m., excluding sucklings) seems to be the highest published for a wild population of any mammal. The small sample size precludes speculation on residue variation at this time with respect to age, season and distribution.

These results are ominous enough to merit further immediate attention to the status of contaminants in the higher trophic levels of the Bay of Fundy food web.

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Earliest Indian Human Remains found in a Late Stone Age Site

THE discovery of human remains from a Late Stone Age site in the village of Sarai Nahar Rai in Uttar Pradesh has provided the earliest available skeletal evidence of man in the Indian subcontinent. This northern Indian site, which is in the district of Pratapgarh, lies about 38 km north of Allahabad city and 33 km south-west of Pratapgarh town, connected by road. It covers about 2,700 m² (about lat. 25° 48' north and long. 81° 50' east) and is about 9 km west of Bishnathganj Railway Station on the Allahabad-Fyzabad line of the Northern Rail-

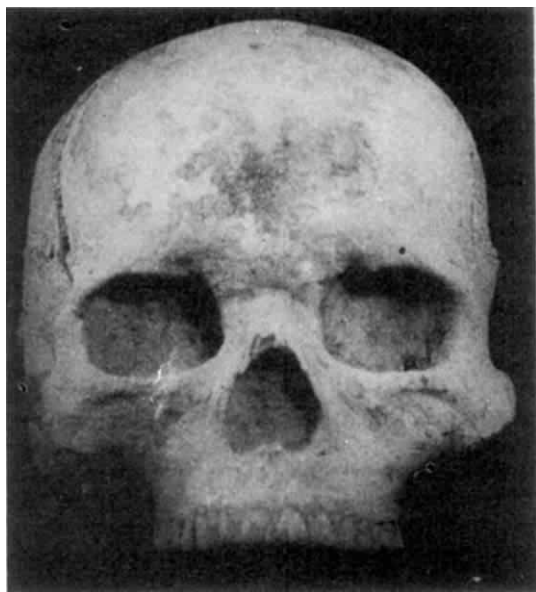


Fig. 1 Norma frontalis of skull of skeleton No. SRN-4 (photograph by courtesy of the Anthropological Survey of India).

way. It was discovered in 1968. The eastern sector of the site—about 600 m²—had been badly eroded and there was visible evidence of a cluster of human graves, the only such example to have been reported from the plain of the Ganga.

A considerable number of microliths and waste flakes made principally of quartz were collected from the surface of the site, where there was a regular microlithic industry. The finished tools, which mostly had steep retouches, included blades, points, piercers, burins, lunates, triangles, trapezes, arrowheads and borers. Heavy tools were conspicuously absent. Chips of charred animal bones were found scattered all over the surface. Some irregular and circular markings on the eroded surface of the site were noticed. These finds represent the debris of a spot where a small group of people had camped.

In March 1970 I conducted an excavation on behalf of the Anthropological Survey of India at the request of the Archaeological Department of Uttar Pradesh, assisted by Mr Anadi Pal. Digging yielded an articulated, almost complete and undamaged skeleton (No. SRN-4) buried in a grave; only the mandible and right humerus were missing. Another eight, badly damaged skeletons were lying nearby, exposed because of heavy erosion of the top soil. The right clavicle and some fragmentary bones of one of these skeletons (No. SRN-5) were removed for examination, and other fragments were sent for radiocarbon analysis to the Tata Institute of Fundamental Researches, Bombay. Some skeletons show evidence of the early stages of petrification.

All skeletons were buried within the deposit of layer 1, and No. SRN-4 was 14 cm below the present surface. This layer is composed of compact and hard silt of a darkish colour. When I revisited the site in May 1971, accompanied by Dr D. K. Sen, director of the Anthropological Survey of India, and Mr P. Gupta, I could locate the skeletal-bearing layer 1 against an exposed section of a nearby small tributary of the Sai River flowing across the district a few kilometres to the north of the site. The layer is about 32 cm thick, resting on an under layer of about 26 cm made up of silt mixed with kankar. Immediately below it is a layer of sandy silt of about 17 cm thick. The nature of the deposit indicates minor climatic oscillations, but generally a dry phase in the post-Pleistocene period.

Clay pots prepared by coiling and baking were found embedded, possibly in some pattern, in most of the graves. The

upper part of most of the pots was, however, eroded away, leaving a circular cross-section of pots projecting from the surface.

There is evidence that the dead were regularly disposed of in a supine posture, usually in a normal extended position with the head to the west (some, however, between west and south but usually within a few degrees of west).

Skeleton No. SRN-4 belonged to a male who was older than 30 when he died. The skull (Fig. 1) is virtually complete and well preserved, and lacks only the mandible. The head is sub-rounded with the keel of the vault somewhat flattened. Seen from the top, the contour is "birsoides". In profile, the forehead is steep, and the superciliary ridges are mesially developed. The face is broad and short with moderately compressed orbits, while the nose is flattish-broad with a marked depression at the base. The mastoids are strong. The cranium has a complete upper dentition which is highly attrited. The wear of the upper incisors clearly indicates that the owner of the skull had the habit of biting in an "edge to edge" manner. The cranial index is 76.04 (mesocranic), nasal index 53.61 (chamaerrhine) and orbital index (left) 74.70 (chamaekonic).

The radiocarbon analysis of a bone sample (index No. TF-1104; depth 5 cm; layer 1) has provided an absolute date of $10,050 \pm 110$ ($10,345 \pm 110$) BP (the first date is based on half life of radiocarbon of $5,568 \pm 30$, and the date parentheses are based on the value of $5,730 \pm 40$ for the half life of radiocarbon)¹.

These remains clearly predate by about 5,800 yr the previous earliest record of man, from the Late Stone Age site at Lekhanua in Uttar Pradesh and dated by radiocarbon.

A detailed report on these finds will be published later.

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¹ Agarwal, D. P., *C¹⁴ Date List—March 1971*, Tata Institute of Fundamental Researches, Bombay (1971).

More about Dowsing

THE article on "Dowsing Experiments" by Foulkes¹ describes some work with which I was concerned and reflects the considerable effort involved in the planning and subsequent analysis of the trial. It does not, however, bring out some of the things which went wrong, which both modify and add to Foulkes's account and which illustrate problems which future experimenters would presumably wish to avoid. My part in this work was the carrying out of the statistical analysis and data presentation which Foulkes reports and it is with these aspects that I am concerned. My remarks are made after reference back to notes I made during the analysis period.

First, the pattern of objects illustrated in Fig. 1 was, indeed, that plan which was originally produced and the objects were nominally laid out in that manner. During some trials with a conventional mine detector, however, subsequent to the dowsing experiment, it was found that row E had not been laid out exactly as planned. Specifically, the objects planned for E6 to E15 were misplaced and actually buried in E5 to E14 and the remaining "blank", planned for E5, was in fact

"buried" in E15. The analysis, which was by this time complete, had to be redone and the Tables given in the later parts of the paper are correct. How this error was not discovered by at least one of the gangs of men who were responsible for burying another class of object is still a mystery.

The results of the two dowers, experimenters six and seven, were not in Figs. 5 and 6 of Foulkes's article. The results of these experimenters were very interesting. They wandered off unsupervised (that is, without an accompanying "caddy" to record their responses). Their results were very similar for both types of ground. The results of experimenter six on the natural ground are given in Table 1.

Table 1 Dowsing Results of Experimenter Six on Natural Ground

Response	Object buried					Totals
	B	M	C	W	P	
M	1	10	2	3	6	22
P	2	11	5	6	7	31
O	1	19	0	1	27	48
(Blank)	36	0	33	30	0	99
Totals	40	40	40	40	40	200

B, Blank; M, metal mine; C, concrete block; W, wooden block; P, plastic mine.

Table 1 has a similar form to Table 2 in the article. On the original score card the dowser was instructed to record M for metallic mine, P for plastic mine and O for nothing present. When questioned about the response O and the blanks left on the score sheet, experimenter seven said that O had been used to mean that an object was present but its identity was unknown and that a blank had been left when a square had been dowsed and nothing found. Table 1 shows clearly a remarkable degree of association between the blank response and the presence of B, C or W and between the O response and the presence of M or P. Since the statement about the meaning of O was made "*a posteriori*" it does not necessarily indicate an ability to dowse and as the two dowers traversed the course together their results are not necessarily independent.

The experiment was therefore repeated by experimenter seven and his results are, in fact, those attributed in the article to experimenter eleven. There was no significant association on this second attempt without experimenter six, who was not retested. This dowser was the soldier illustrated in Fig. 3 of the article, a Nigerian, who unfortunately could not be retested. These results, although inconclusive because of the lack of experimental control, do suggest that something peculiar occurred on this occasion which might be worth further investigation. Two different conclusions, all too common in this type of work, can be drawn. Either some form of ability was demonstrated (although peculiarly recorded and interpreted) or a breach of security of the trial (including the possibility of cheating) took place. It can be argued that it would be possible for an unsupervised student to have prodded the ground (for example with one of the dowsing rods) and at least detected an object present, since for control purposes small wooden pegs indicated the position in which an object, if present, would have been buried. Although this form of cheating may well have taken place on this occasion it does not explain how mines and other objects might have been differentiated. Indeed, since the basic concern was with methods of detecting underground mines, a proven ability to detect the difference between a mine and an object (for example a stone), even by prodding, could have been of considerable importance.

There is also slight evidence that a positive response was more closely associated with the presence of a wooden block than with a concrete block—especially on the raked ground. Although this may be a spurious result, the wooden block may

have absorbed more moisture than the concrete block, thus altering the drainage pattern and hence the surface appearance of the ground.

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¹ Foulkes, R. A., *Nature*, **229**, 15 (1971).

Dowsing Experiments Criticized

My photograph appeared in Foulkes's article, Dowsing Experiments¹, without prior reference to me, and I would like to make the following comments on the article.

The experiments carried out at the Military Engineering Experimental Establishment (MEXE) did not involve true "dowsing" as they were not water divining. Although experienced dowers can locate buried objects such as cables, pipes and the like, these experiments were a search for objects unfamiliar to those taking part. Those I spoke to made no claim to achieve success, but agreed to the trial as an interesting experiment that might be of practical use. It was also evident to all that, to be of practical use, map dowsing must be used when walking over a live mine field. I agree that this experiment was a failure although one man was successful far beyond the bounds of chance.

The "flow in a pipe" experiment is not one which I would attempt, although I could probably locate the pipe either full or empty. No mention is made of the dowser's experience in this particular kind of search. I consider this test valueless, if he had no such experience.

I would like to emphasize that I was not instructing anyone in dowsing during the exercise at Chatham. The object was to find a number of men who could usefully develop into proficient water diviners. I do not think it possible to instruct anyone. Of those involved, 10%, not 25%, found accurately my second flow line; their judgment coinciding with mine and confirming my classification of them as sensitives.

I still maintain that the well referred to was correctly sited on one of the flows in the upper chalk. I predicted that the water would be near the bottom of the upper chalk and detectable by drilling through the clay to the chalk. This has not been done, as the drilling rig was removed before it had passed through the clay stratum. This was, therefore, not a dowsing failure.

The test over the 42 inch pipe seems to have been made by some of the 10% of "sensitives", who had no real dowsing experience. Even over natural fissure flows I would not have expected these novices to assess quantity.

Foulkes's description of the V rod movement was inaccurate. If the rod is held firmly in the normal way, another person can press the tip up or down, and, when released, the rod will return to its original position without any additional effort by the holder.

The Rocard experiment proves nothing. There are many dowers who cannot dowse in rubber soled shoes. If Rocard, thinking he had used magnetized iron in his elbow joints, actually used non-magnetic iron, the experiment could have had the same result. It is a pity that Foulkes does not seem to have consulted any other authority than Rocard.

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¹ Foulkes, R. A., *Nature*, **229**, 15 (1971).

BOOK REVIEWS

Social Biology

Social Implications of Biological Education. Edited by Arnold B. Grobman. Pp. 134. (Darwin: Princeton, New Jersey, April 1971.) \$5.95.

THIS book was planned by a Committee of the American National Association of Biology Teachers for those who would welcome thoughtful reviews of pressing social problems associated with advances in the biological sciences. It is the report of a national conference of NABT held in 1969, in which papers prepared by distinguished biologists, who have exhibited a concern for significant social problems, are followed by the prepared statements of three panelists, which support, contradict, extend or restrict the major papers. The three members of each panel include at least one additional specialist in the field of the major speaker and one practising school teacher. The statements of the panels are followed by accounts of the question and answer sessions.

Four major topics, medicine, behaviour, genetics and population, are dealt with in this manner, and, owing to Dr Grobman's skilful editing, this technique has produced a lively and readable volume. Such questions as "Should the government support and control scientific research?", "Should euthanasia be practised?", "Is free-will an obstacle to technological progress?", "Are differences of intelligence a matter of race?", "Is birth control a solution to the population problem?" and "Should man control his genetic resources?" are ably discussed from differing viewpoints.

The section on medicine is introduced by a paper on "The Social Implications of Medicine" by Michael and Lois Debakey of the Baylor College of Medicine, Houston, Texas, who discuss the growing discrepancy between the achievements of research and technology and their application to human life. The importance of the science teacher's role in conveying not only basic concepts, the philosophy and methods of science, but also the social implications of medical science, so that each student may recognize his responsibility in helping to shape public policy in this field, is clearly brought out.

James V. McConnell of the University of Michigan introduces the topic of behaviour in a controversial paper on "Ethics and Behaviour", in which he discusses life from a behaviourist's standpoint, and a paper by Bruce Wallace of Cornell University entitled "Genetics and Genetic Manipulation" opens the section on genetics. This

latter gives an interesting treatment of biological races, and comments on recent work on "racial intelligence", as well as considering eugenics and genetic engineering. This is followed by a stimulating paper entitled "Can Teachers Tell the Truth about Population?" by Garrett Hardin of the University of California.

The last chapter departs from the pattern adopted for the rest of the book. It is based on the banquet address by Claude Welch on "Evolution—the Reluctant Revolution", in which the nature of conceptual revolutions is discussed.

This volume is not intended to be a complete compendium of the social implications of biological education. It is selective and omits some very important problems. For instance, there is no section devoted to ecology, and the whole vital area of the control of pollution and the redressing of the environmental balance is only hinted at. The reason for this omission might well have been that one of the few social problems, which American biology teachers of a generation ago thought appropriate for their consideration, was conservation. In this area, NABT played an active part and, under the leadership of the late Dr Richard Weaver, the Association produced a *Conservation Handbook*, which has been highly regarded.

Furthermore, the treatment of those topics, which are dealt with in this volume, does not set out to present all possible points of view. The aim is rather to present a number of thought-provoking and related statements on current problems for teachers to consider. Where the chosen topics have implications, which fall into the two broad areas of social-political and moral-ethical, it is in the former area rather than in the latter that problems are explored.

This should be a particularly useful book for teachers of the life sciences, who wish to discuss with their students those "problems generated at the interface between science and society". As science becomes broadly integrated into all phases of our culture, its significance as a part of a general education becomes more important and the aims of science teaching will need to go beyond the restrictive context of the special disciplines and consider science in relation to mankind. It is clear from the discussions reported in this volume that it will meet a need for science teachers who are now expected to take on an entirely different kind of responsibility from that for which they have received preparation. ELIZABETH PERROTT

Parasitic Insects

Parasitic Insects. By R. R. Askew. Pp. xvii+316. (Heinemann: London, June 1971.) £3.50.

THIS book is divided into two sections; the first deals with insects having parasitic adults and the second with protean parasitic insects. The author adopts a very broad view of parasitism and a wide range of diverse associations are described under this umbrella definition. The first section is introduced by a description of the mouth parts of adult parasitic insects, which possibly would have been more easily comprehensible had it been treated from the point of view of adaptive radiation and with a little more emphasis on function and the physiology of digestion of imbibed host tissue. Some treatment on the ecology of the host body environment and the alterations (for example, the histopathological changes) inflicted on it by the parasite would have been useful and may even give a lead to explain the absence of anticoagulants in the saliva of some of these species. For a parasite does establish a physiological association with the tissues on the surface of, or inside, the body of its host, even though the association is primarily to provide food for the parasite. This omission is not altogether the fault of the author, for much remains to be done in this field.

Chapters 2–5 deal with lice, fleas, blood sucking flies and Diptera pupipara and chapter 6 with bugs, earwigs, beetles and moths as a "convenient" grouping of other insects parasitic as adults. I believe that the bugs should have been dealt with in a chapter on their own. The approach to these organisms is from the ecological standpoint and there is undoubtedly a wealth of information here, with substantial reference to up to date literature. The final chapter in this section deals with blood sucking insects as vectors of human disease, but more could have been done on this aspect of the subject—for example, some reference to the factors influencing the movement of trypanosomes in the insect gut and the passage either to the proboscis or to the hindgut preparatory to transmission. The insect body is, after all, an environment for these pathogens and they must show physiological adaptability to it. In spite of these apparent criticisms there is no doubt that the author has drawn together much information of considerable value for undergraduate students and as introductory reading for post-graduates specializing in ectoparasites. In the introduction (page X) the

author states that the term parasitoid will not be used in the book, but he proceeds to do so on page 111! The author is obviously very much more at home when writing on parasitic Hymenoptera (chapter 8) and presents an excellent review of their structure, function and ecology extending over seventy-three pages (compared with ninety-four pages for all insects with parasitic adults). Thirteen families of protean parasitic Diptera are likewise covered in some detail and these chapters are eminently suitable for post-graduate reading. The characteristics of biological control of insect pests form the substance of chapter 10. Fly larvae which parasitize vertebrates (chapter 14) might appropriately have been included in the first section.

This book fulfils a real need for a variety of students, it is well composed, is illustrated with clear line drawings of the highest quality, and the whole is completed by a substantial list of relevant references.

DON. R. ARTHUR

Life on Earth

The Nature of Life: Earth, Plants, Animals, Man and Their Effect on Each Other. By Lorus Milne and Margery Milne. Pp. 320. (Cassell: London, August 1971.) £5.50.

Lorus and Margery Milne have produced a beautifully illustrated book about the life on this planet. It is not a technical book, but the text is informative and full of interesting miscellaneous facts, for example, that the shell of the foraminiferan *Globorotalia* coils clockwise when the climate is warm and anticlockwise when it is colder and that this has enabled geologists to obtain a measure of the temperature of the sea at different times.

The book covers all the continents of the world as well as the oceans, describing the geology, climate and forms of life to be found there. It would have been greatly improved by the use of good maps of the places being described. As it is, the almost complete absence of any maps means that one is constantly coming up against the names of faraway places, the locations of which are only dimly remembered from school geography.

The final chapter is about the damage which the human species has done and is continuing to do to its environment. Although the message is now a commonplace one, it is difficult not to be impressed all over again by the fact that one species of mammal and one species of bird become extinct, on the average, every eight months, often as the direct result of human activity.

The Milneses should succeed in inducing in the reader the desire to visit and

explore as they have done and to see for himself the beautiful and varied faunas they describe.

MARIAN DAWKINS

A Young Science

An Introduction to Psychopharmacology. By Richard H. Rech and Kenneth E. Moore. Pp. xii+353. (Raven: New York, 1971. Distributed in the Eastern Hemisphere by North Holland, Amsterdam.) £4.65.

Psychopharmacology and the Individual Patient. Edited by J. R. Wittenborn, Solomon Goldberg and Phillip R. A. May. Pp. 256. (Raven: New York, May 1971. Distributed in the Eastern Hemisphere by Pitman Medical and Scientific, London.) £5.50.

PSYCHOPHARMACOLOGY is somewhat isolated from disciplines such as neuropharmacology, psychophysiology and neurochemistry and is not yet integrated with its most relevant basic sciences, pharmacology and experimental psychology. Only psychiatry has developed a common area with psychopharmacology, that of drug therapy in mental illness. The reasons for this state of affairs which continues nearly twenty years after the discovery of the first of the modern psychotropic drugs, chlorpromazine, lie in the recent historical development of the subject. Most, if not all, of the important new psychotropic drugs were discovered by serendipitous accident. Thus, chlorpromazine and imipramine were first noted to have potentially useful actions in patients. Psychiatrists using these drugs on the wards and in their out-patient clinics quickly realized that real therapeutic advances were being made with essentially new drug types. Pharmacologists and psychologists then sought actions of these drugs both in animals and in normal humans which would provide some rationale for their therapeutic effects.

In spite of all the research effort of the past two decades, the mode of action of most psychotropic drugs remains obscure or, more strictly speaking, the clinical relevance of the pharmacological actions which have been found remains unproven. For example, for some time after the introduction of the tricyclic anti-depressants, their mode of action was unexplained. Eventually it was found that they inhibited the reuptake of noradrenaline from the synaptic cleft. The other class of anti-depressants, the monoamine oxidase inhibitors, had from the start been assumed to owe their clinical action to that implied in their generic name. However, it now seems possible that they, too, inhibit noradrenaline uptake and that their enzyme-inhibiting properties may be less relevant.

For these and many other reasons, psychopharmacology has remained a loose federation of disciplines rather than a coherent subject, and at the clinical level it is probably the most empirical branch of therapeutics. These two multi-author American books represent two different approaches to the problem of maturing psychopharmacology from the empirical to the rational. *An Introduction to Psychopharmacology* aims at providing for the neophyte background reading in cognate subjects. Two rather breathless chapters attempt the impossible task of outlining pharmacology, psychology, neuroanatomy, and neurophysiology in eighty pages: there are many basic texts more suitable for this purpose. The remaining chapters are much more successful, covering biochemical aspects, the limbic system, electrophysiology, conditioning and animal testing of new drugs, with two clinical chapters on tranquillizers and anti-depressants. Except for the chapter on anti-depressants which is in the "in my experience" style, the scientific standard is high and the material presented is critically appraised. Useful key references and reviews are included. The chapter by K. E. Moore on "Biochemical correlates of behavioral effects" is very balanced and avoids the overstated claims of those who believe that biochemical mechanisms in adult mental illnesses are already apparent.

Psychopharmacology and the Individual Patient is disappointing and will be of interest to very few people. It comprises the proceedings of a meeting of the Prediction Study Group of the American College of Neuropsychopharmacology held in December 1968. The object of the research projects described in this book is to predict which patient will respond to which drug. A typical study follows this recipe: take about 500 patients, measure them on about seventy-five variables, clinical and demographic, treat them with one or more drugs and assess the outcome. Use complicated statistics to see which variables influence the outcome. The results are not only indigestible but only about twenty per cent of the outcome variance can be predicted, rendering the exercise unproductive. One reason for this failure may lie in the neglect by the investigators of pharmacological factors. Only in the excellent introductory chapter by S. Gershon is attention drawn to the marked variation between patients in plasma levels of many psychotropic drugs. The reluctance of many investigators to use pharmacological techniques in clinical studies will perpetuate the isolation of psychopharmacology which will be unfortunate as such techniques promise to rationalize our clinical use of psychotropic drugs.

MALCOLM LADER

Clinical Enzymology

Mattenheimer's Clinical Enzymology: Principles and Applications. By Hermann Mattenheimer. Pp. xiv+168. (Ann Arbor Science: Ann Arbor, May 1971.) \$14.75.

Enzyme Synthesis and Degradation in Mammalian Systems. Edited by M. Rechcigl. Pp. xvi+477. (Karger: Basle, Paris and London. Distributed in the USA and Canada by University Park: Baltimore, 1971.) 90 Sw. francs; £9.50; 90 DM.

MEASUREMENTS of the activity of enzymes in blood serum constitute one of the most important ingredients in the explosive growth of clinical biochemistry. Beginning as a series of random observations of changes in the levels of a few serum enzymes in a limited number of diseases, diagnostic enzymology now offers systematic data of wide clinical application, based on rational hypotheses about factors which influence the levels of enzymes in the blood: this is particularly so with regard to the leakage of intracellular enzymes which accompanies acute tissue damage. However, efforts to assess the nature of intracellular events by direct measurement of enzyme activities in tissue samples have made slower progress, mainly for methodological reasons, except in the demonstration of inherited conditions which affect the metabolism of red blood cells and in certain other metabolic abnormalities. The growing importance of clinical enzymology has been accompanied by the appearance of textbooks which aim to codify and to rationalize the mass of data which appears yearly in the medical literature, and to guide clinicians and clinical biochemists in the scientific basis, selection and interpretation of enzyme tests. Mattenheimer's book fulfils this function, and in addition offers some differences in content and emphasis from other books in this field. Thus, only one chapter of twenty-five pages is devoted to interpretation of enzyme tests on blood plasma, with a further eight pages on enzyme tests on urine. The other eight chapters are devoted to brief descriptions of principles of enzymology, metabolic pathways, enzyme-hormone interactions, protein synthesis, inborn errors of metabolism, and the enzymology of cells and organs (two chapters). The last two topics, with their implications for future developments, form perhaps the most interesting and original part of the book. The subject of the last chapter, methods of estimation of enzyme activity, could have been left to specialized laboratory handbooks.

This wide range of topics inevitably results in superficial treatment of some; for example, protein synthesis and biochemical genetics, and intermediary

metabolism, but the value of the book lies mainly in its correct emphasis on the unity of clinical and non-clinical enzymology. There are a few errors and omissions: alkaline phosphatase activity is probably not decreased in the intestine in hypophosphatasia (page 99); the statement that bone metastases of cancers other than prostatic carcinoma are not accompanied by elevated plasma acid phosphatase (page 128) is incorrect, and in the discussion of inborn errors of metabolism, some reference to lysosomal enzyme deficiencies in lipid storage diseases and the role of amniocentesis in prenatal diagnosis would have added an up-to-date touch. These minor criticisms should not, however, deter those concerned with clinical enzymology, especially clinicians, from reading the book.

Interpretation of enzyme activities in organs or blood is related fundamentally to a knowledge of rates of enzyme turnover and of factors which influence them. *Enzyme Synthesis and Degradation in Mammalian Systems* is a valuable collection of twelve reviews of research on these topics, particularly by reason of its mammalian, rather than microbiological, emphasis. The chapters vary in length from fifteen to sixty pages, but all are well written and are supported by extensive lists of references: these include a number dated 1969, so that the picture is essentially contemporary. The relative value of individual articles will be assessed differently by different readers, depending on their own familiarity with the topics discussed. However, the long chapter by Rechcigl on enzyme turnover is particularly comprehensive, and brings out clearly the prevailing state of mystery with regard to mechanisms of enzyme breakdown. The apparently highly selective nature of the processes involved in enzyme turnover is well exemplified by the case of lactate dehydrogenase isoenzymes (reviewed here by Vesell and Fritz), in which rates of synthesis and degradation in two catalytically and structurally similar proteins are found to differ widely within a single type of cell. Gelehrter argues cogently against the uncritical translation to mammalian systems of data on the induction of enzymes in microorganisms, while chapters on nutritional (Freedland) and hormonal (Rosen and Mulholland) influences on enzyme activity, and its rhythmic variation (Fuller), also emphasize the complexity of enzyme control in the whole animal.

These chapters, and the others in this collection, constitute a stimulating volume but, in view of the problems which await solution, one which also cautions against over-optimism. Perhaps, however, only a few years will elapse before reviews of this nature will need to warn against too ready an extrapolation of mechanisms established for

rat liver to processes of human health and disease.
D. W. Moss

Nuffield Texts

Nuffield Science Teaching Project: Advanced Science (Chemistry). Special Studies (ed. J. G. Raitt): Food Science. Pp. 150. 90p. Biochemistry. Pp. 150. 90p. *Programmed Text: Ethanol and other Alcohols.* By Erica Glynn. Pp. 137. 60p. *Standard 8 Film Loops: Born-Haber Cycle; Hydrolysis of Bromoalkanes; Rates of Reactions.* £6.30 each. Penguin Education, Penguin Books Ltd; Harmondsworth, Middlesex.

THIS latest batch of Nuffield A-level chemistry materials maintains the generally high standard of its predecessors. *Food Science* is clear, direct, and stimulating; I learnt a great deal from it. The social aspects of the study are never kept in the background, and a major aim of the book is stated on page 3: "... The broad outline of the subject may help (readers) to consider how an understanding of the chemistry, physics and biology of foods can contribute towards the improvement of the health and well-being of mankind, and towards solving the problems of the shortage of food in the world". Nonetheless, the chapter on the world food problem fails to go far enough in posing the intense problems which must be faced within this century. The book is particularly good on technology, and there is an excellent outline of the processes occurring in foods during treatment and storage. The authors fire a salvo at macrobiotic food fanatics (page 95) in the course of an interesting chapter on food legislation and public health. A-level readers may find some of the chemistry tough going (for example, the browning reaction on page 56); but the book concludes with a good practical section.

Biochemistry seems to be written in a less easy style, although it gets much better as it goes on. It starts badly with some facile comments on DNA (page 1) which include a disturbingly naïve paragraph on genetic engineering, and a silly question about the Golgi apparatus (page 9). Assumptions are made about the "vocabulary" of A-level readers, particularly in the section on metabolism; here, Lehninger's diagrams are not sufficiently clear to give confidence to those treading these complex pathways for the first time. Able pupils may wonder why (as in *Food Science*) monosaccharide hexoses are always represented by Haworth structures rather than in the chair conformation, and why the stereochemistry of, for example, cholesterol and gibberellic acid is not shown. Fig. 49 has a mislabelled axis; the caption for muscarine is mysteriously transposed from page 51

to an innocent amylopectin chain on page 53.

The book has several excellent sections, especially on medical aspects. The agricultural section is fine, but the dangers of pesticide abuse are not mentioned. The chapter on biochemistry and commerce is compelling, particularly the section on sewage disposal. (I was intrigued to see a photograph of our local rural district council's activated sludge plant.) The experimental investigations look good.

Ethanol and other Alcohols is a straightforward, highly competent programmed text containing a large proportion of practical work.

The film loops are much better than their O-level counterparts. They limit themselves to one topic and work at it thoroughly. The accompanying notes are very lucid. *Rates of Reactions* provides a convincing demo using the Dainton/Fisher ping-pong ball machine. *Hydrolysis of Bromoalkanes* makes good use of models, and the diagrams in the *Born-Haber Cycle* are bright and clear. My only criticism is the cost of these films.

MARTYN BERRY

X-Ray Diffraction

Interpretation of X-ray Powder Diffraction Patterns. By H. Lipson and H. Steple. Pp. viii+335+3 plates. (Macmillan: London; St Martins Press: New York, May 1970.) £4.

THE textbook *Interpretation of X-ray Diffraction Photographs*, by Henry, Lipson and Wooster, itself the distillation of much teaching experience, has been a standard reading and reference work for many generations of undergraduates and of postgraduates learning to use crystallographic techniques. But since it was first published the subject has developed enormously and new methods, both experimental and interpretative, have become both available and standard.

To have revised the original book would have been to produce a textbook both too unwieldy and too expensive; and the decision was therefore made to rewrite the book in separate parts, making each complete in itself. For the practical study of many important solid materials that do not form single crystals of adequate size for individual examination, or that can only be obtained as a mass of randomly oriented crystallites less than, say, 10 μm in individual size, powder diffraction methods are essential. Moreover, X-ray photographic methods have been supplemented or, for some purposes, replaced by counter-diffractometer techniques including those of neutron diffraction. To the metallurgist and the solid state physicist, the geologist and those concerned with phase diagrams of all kinds,

or simply with the identification of unknown materials, the powder diffraction pattern is invaluable. The technique is, in general, non-destructive and requires less than 1 mg of material; or it can be used with massive specimens *in situ*. It is therefore of the utmost practical importance. The preface implies that this is an undergraduate textbook: but it is much more than that.

Nevertheless, it is aimed in the first place at undergraduates and it begins therefore at the beginning, with crystal lattices and crystal symmetry, with the geometry of X-ray reflexion and the nature and production of X-rays. Some of the diagrams in this section are less than satisfactory. Fig. 3.2 would give a student a very weird idea of the spectrum from a copper target run at 35KV, and fig. 3.6 that of both the intensity of radiation and absorption of very short wavelength X-rays. These are followed by methods of recording and measuring powder patterns and of interpretation, with descriptions of standard and special-purpose equipment. It is a pity that although measurement of coefficients of thermal expansion is mentioned as an application, nothing is said about the absurdity of claiming to make accurate dimensional measurements (a limit of one part in 100,000 is mentioned) unless the temperature of the specimen, and not merely the ambient temperature, is measurable and is recorded.

The chapters on applications make one wish for more.

A new feature is the section of undergraduate problems (with solutions), but there follow the useful appendices and tables, with subject, references and author indexes that will certainly extend the use of the book beyond that of the undergraduate. KATHLEEN LONSDALE

(This review was completed by Dame Kathleen Lonsdale before she died earlier this year.)

Analysis by Activation

Principles of Activation Analysis. By Paul Kruger. Pp. xi+522. (Wiley: New York and London, August 1971.) £11.75.

RADIOACTIVATION analysis, a by-product (or should it be a spin-off to be really up to date?) of research in nuclear science, is now well established in its own right as an important tool in science and technology. In this book, Kruger discusses some of the applications in his final and longest chapter and devotes a good fraction of it to the analysis of trace elements, an appropriate choice in view of the very high sensitivity of activation analysis. In this day and age, industry and the environment were almost bound to be mentioned, and they are, but sensibly and with reasonable detail. Oxygen in metals, the logging of oil

wells and on-line techniques are the main examples from industry; here, as in the other fields which I do not have the space to cover, the examples are clothed with extensive numerical information.

It is important for materials analysts, particularly those with a background in nuclear physics, to realize that the nucleus is not the only part of the atom capable of emitting identifying radiations, as I am sure the optical and X-ray spectroscopists would quickly point out. With this in mind, I was pleased to see that Kruger's section on trace element analysis includes a comparison of activation analysis with other methods; the electron probe is not among the others but, even so, the reminder to think broadly is there.

These comments have arisen just from Kruger's final chapter, but what of the rest of the book? The three chapters on the practices and limitations of activation analysis and on radiochemistry, which together form more than a third of the whole, are thorough and should be very helpful to the serious student of the subject. How to select the stimulating radiations, how to use the stimulated ones, whether to include chemical methods, how computers can help—these are the broad issues and in each case there is enough information to satisfy the budding professional. Here he can learn about the complexities of gamma-ray spectra, the problems of spectral stripping, the ways to deal with interfering reactions and a number of other features, and at the end of each chapter he has a few problems to sharpen his teeth on.

This, then, is a book with much useful information, and it is elegantly produced with clear diagrams and tables, but the cost is high for the individual buyer, at least in the United Kingdom. There is no doubt that half the book is well worth half the cost, but less introductory material and a lower total cost would have been a better balance, in my view, because this would have made the book more widely available. After all, Kruger's own excellent bibliography makes it clear that there are already several texts covering this first part of the work. Moreover, cuts in this section could have removed the few slips and ambiguities which are there (resonance scattering of neutrons is not synonymous with inelastic scattering; the relationship between thermal neutron fluxes and fast neutron yields is not as simple as implied; a conventional neutron flux, not the one defined, must be used with tabulated cross-sections to obtain reaction rates; in a Maxwellian distribution at room temperature, 0.025 eV is the energy associated with the most probable speed, not the most probable energy).

JOHN WALKER

CORRESPONDENCE

Italian Science

SIR,—In Italy, research is almost completely in the hands of the National Council of Research (CNR), founded by Mussolini on November 18, 1923 (see *La Ricerca Scientifica*, 1945, p. 108). According to OCSE's experts, the CNR can be compared—as far as its juridic nature is concerned—to “an institution with a corporative nature, comparable with those professional orders which have multiplied during the fascist period”. At present all financing has been suspended, and many applications have been rejected, even when some months ago it seemed that they had been approved. As for the applications which have been approved, the funds are still to be found on the budget of 1970 and some say that money for the research going on at present will not be paid until next spring. Young scientists and technicians are therefore obliged to abandon the universities, and projects (some international) must be cancelled in our programmes (supposing one may speak of “programmes” in Italy). And unfortunately this is not the first time we have had serious administrative trouble, though the CNR has at its disposal more employees than research workers.

One example is that of the International Biological Programme (or IBP), which for us was the first timid approach towards ecology, more especially productivity: whereas in other countries the IBP has seen and is still seeing beautiful research, which will go on until 1972, in Italy they say it is over. Whereas abroad many contributions have been published in the field of IBP, here there has been a very poor production. Even the results of the exploration of the small Italian islands, included in the CT programme of IBP, are extremely poor.

This contrasts strongly with the results Italian scientists had achieved in the field of the International Geophysical Year (1955), only 10 years after World War II. We cannot help thinking therefore that our Government's scientific policy is fundamentally very bad.

Useless to say that this has been well known abroad for several years: suffice it to quote Di Ferrante (*Science*, **161**, 451; 1968), Consolazio (*ibid.*, 133; 1968), Greenberg (*ibid.*, **167**, 1706; 1970), and Survey of Science in Europe (*Nature*, **226**, 1016; 1970) who give a very clear and exact description of Italian scientific policy, especially in calling the members of CNR “self serving advisers” (Di

Ferrante, p. 451). We must not forget, however, that already in 1955 a member of CNR, Professor Di Mattei, had made a very clear critique of its policy, in which he says (*La Ricerca Scientifica*, **25**, 2372; 1955) “the Committee (for Biology and Medicine) has over all limited itself to a mere paternalistic distribution of the funds it had at its disposition, and has not confronted the great scientific problems which should have been the highest task of its financial policy”.

What our responsible men are not able to do is to split *completely* the research in the universities (which are entirely state entities, depending on the Ministry of Education) and that in the CNR centres (which depend on the Presidency of Ministers), keeping two independent budgets, with no interchange or osmosis possible between the two. The full separation of powers and of men would be particularly necessary in a country such as Italy, which is famous all over the world for its “camarillas”, mafia, “Baronie”, clientelism, and so on. Of course the stronger the relationships between university and CNR, the easier the clientelism between the “owners” of CNR and the university institutes, which today depend for research completely on the former because practically all the members of CNR (presidents of committees, sub-committees, work groups, sections, and so on) are also professors in the chair at some university. With the last events of the CNR, the fear that all of us (university workers) have to be turned in post-lyceum teachers instead of research workers is stronger and stronger and quite justified. On the other hand, the political situation of the country (see a series of articles by P. Nourry and P. Bois in *Le Figaro*, the last of which was published on September 22) does not permit any sort of optimism in this field.

Yours faithfully,

GIORGIO MARCUZZI

The University of Padova

Suckling Etymology

SIR,—In his letter (*Nature*, **233**, 73; 1971) Mr Spratling complains that a communication¹ which several co-signatories and I sent to you some 20 years ago has been the cause of the confusion he has recently experienced in fairly extensive reading on the topic of suckling. We are apparently to blame because we used the verb “to suckle” in a manner contrary to the usage

of Shakespeare and of that found in the Bible.

English usage never stands still. It is nearly 300 years since the verb “to suckle” began to be used to denote the activity of the young as well as that of the lactating mother. This extension of meaning has caused ambiguities. It was because of this long-standing ambiguity that the signatories of the letter, who were then all working in the field of lactational physiology and well aware of the difficulties that could arise, suggested that it might be helpful if authors of scientific papers were to restrict the use of this verb to denote only one activity and since its commonest usage in contemporary papers was with reference to the activity of the young we suggested that it be so restricted. Mr Spratling is, of course, equally entitled to suggest that the meaning be restricted to the activity of the mother provided that he indicates, as we did, that he uses the word in a restricted sense. To imply, however, that his choice is the only correct usage is, at the present time, nonsense. He should get an up to date dictionary and put Shakespeare on the shelf.

In Mr Spratling's description “the calf sucks the cow” the use of the verb “to suck” is unfortunate, not in this instance because of any change in usage since Shakespeare's time but because there have been some advances² in our understanding of the process whereby the young ruminant acquires milk from the udder of its dam. The movement of the suckling's tongue against the hard palate exerts positive pressure on the teat, stripping the milk from it in a manner similar to that of the fingers of the hand milker, suction being a non-essential component of the process. Only the milking machine which obtains milk from the cow solely by the creation of a partial vacuum may, I think, truly be said to suck the cow.

Yours faithfully,

A. T. COWIE

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Reading RG2 9AT*

¹ Cowie, A. T., Folley, S. J., Cross, B. A., Harris, G. W., Jacobsohn, Dora, and Richardson, K. C., *Nature*, **168**, 421 (1951).

² For references see review by Cowie, A. T., and Folley, S. J., in *Sex and Internal Secretions*, third ed. (edit. by Young, W. C.), **1**, 627 (Williams & Wilkins, Baltimore, 1961).

British Diary

Saturday, October 16

Unemployment Among Scientists and Technologists (10 a.m.–6 p.m.) British Society for Social Responsibility in Science, at the Harkness Hall, Birkbeck College, Malet Street, London WC1.

Tuesday, October 19

Extra-Sensory Perception: Fact or Fallacy? (1.30 p.m.) Dr A. J. Ellison, University of London, at Queen Mary College, Mile End Road, London E1.

Insight, Not Numbers (5.30 p.m.) Professor P. A. Samet, University of London, in the Chemistry Auditorium, University College London, Gower Street, London WC1.

New Techniques for the Determination of the Nutritive Value of Feeding Stuffs for Ruminants (10.30 a.m.) Society of Chemical Industry, Agriculture Group, at 14 Belgrave Square, London SW1.

Problems of Large Power Stations (7.30 p.m.) Institution of Chemical Engineers, jointly with the Institution of Mechanical Engineers, at the University, Nottingham.

The Tectonic and Depositional History of the Eastern Equatorial Pacific During the Cenozoic (5.30 p.m.) Professor Tj H. van Andel, University of London, in the Collegiate Theatre, University College London, Gower Street, London WC1.

Vinyl Resins in Surface Coatings (7 p.m.) Mr D. J. Silsby, Oil and Colour Chemists' Association, at the Pen-dragon Hotel, Southsea, Hampshire.

Wednesday, October 20

Application of Satellite Relayed Communications to Civil Aviation and

Maritime Use (6 p.m.) Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Biomedical Research and the Chemist—a View and a Prospect (5 p.m.) The Rt. Hon. the Lord Todd, FRS, at the Royal College of Physicians, 11 St. Andrews Place, Regents Park, London NW1. (Merck Sharp and Dohme Research Laboratories Scientific Lecture.)

Computer-Aided Design in Electronics (5.30 p.m.) Mr P. E. Trier, Institution of Electrical Engineers, at Savoy Place, London WC2.

Developments in Water Based Surface Coatings (7.30 p.m.) Mr A. J. Becalick, Oil and Colour Chemists' Association, at the Carlton Hotel, North Bridge, Edinburgh.

Electronic and Radio Needs for Light Aircraft (6 p.m. discussion) Royal Aeronautical Society, at 4 Hamilton Place, London W1.

Engineering for Nuclear Power (10 a.m., Mr J. C. C. Stewart, Dr D. F. Seymour) Dr Gordon Brown and Mr P. H. W. Wolff, in the Faculty of Applied Science, University of Nottingham. (Bulleid Memorial Lectures.)

How to Survive on the Planet Earth (6.15 p.m.) Dr A. Peccei, United Kingdom Automation Council, in the Council Chamber at the Confederation of British Industry, 21 Tothill Street, London SW1.

Martin Lister (6 p.m.) Mr S. Peter Dance, Society for the Bibliography of Natural History, in the Men's Staff Common Room, University College London, Gower Street, London WC1.

Speech Scrambling and Security (7.30 p.m.) Mr R. C. French, Institution of Electrical Engineers, at the Mullah Research Laboratories, Redhill, Surrey.

The Training of Sandwich Course Students on Degree Courses Containing Options in Analytical Chemistry (3 p.m. discussion meeting) Society for Analytical Chemistry, Education and Training Group, at the Thames Polytechnic, Woolwich, London SE18.

Thursday, October 21

Agricultural Productivity in the Third World (8.15 p.m.) Professor Sir Joseph Hutchinson, University of London, at Wye College, near Ashford, Kent.

Pollution (7 p.m.) Dr R. B. Brown, Oil and Colour Chemists' Association, at the Beech Tree Hotel, Maxwell Road, Beaconsfield, Bucks.

The Evolution of the Geosyncline Concept (5.30 p.m.) Professor Ken Hsu, University of London, in the Collegiate Theatre, University College London, Gower Street, London WC1.

What Strings on Medical Research? (5.30 p.m.) Sir John Wolfenden, University of London, in the Beveridge Hall, Senate House, London WC1.

Friday, October 22

The Distance Scale of the Galaxy (9 p.m.) Sir Richard Woolley, FRS, Royal Institution, at 21 Albemarle Street, London W1.

The Paleomagnetism of Marine Sediments (5.30 p.m.) Dr N. D. Opdyke, University of London, in the Collegiate Theatre, University College London, Gower Street, London WC1.

The Quenching of a Diazo-Chromophore Fluorescence by Aliphatic Hydrocarbons (1 p.m.) Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

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